GROWTH AND POST-HARVEST QUALITY OF SELECTED PACIFIC OYSTERS (*Crassostrea gigas*) CULTURED IN KACHEMAK BAY, ALASKA, AND PUGET SOUND, WASHINGTON, IN OCTOBER 2009 AND JUNE 2010

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A

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Abstract

The primary objective of this project was to evaluate the growth, biochemical and fatty acid composition, physical and shell characteristics, and basic reproductive development of families of Pacific oysters (*Crassostrea gigas*) from the USDA-funded Molluscan Broodstock Program (MBP) planted in suspended culture in Kachemak Bay (KB), Alaska, and at an intertidal site in Thorndyke Bay (TB), Puget Sound, Washington. The MBP selects oysters to improve yields, growth, and survival, but little is known about the effects of selective breeding on other biological characteristics of selected oysters. Shell and meat characteristics of oysters from each of the seven highest-yielding MBP families were compared with those from non-selected control families at each site, which were sampled in October of 2009 and in June of 2010. Biometric and growth data, proximate compositions, fatty acid compositions, and basic degree of reproductive development were measured and compared by family, site, and sampling time. Selection improved yield, growth, and survival in MBP Cohort 20 oysters over three years of growout at KB. Colder water temperatures at KB relative to TB inhibited reproductive development, altering the biochemical composition of oysters within sites and between sampling times. Oysters grown at KB were slower growing and smaller when compared to TB, but higher in glycogen, ω-3, and ω-6 fatty acids (particularly docosahexaenoic acid: 22:6ω3). Different latitudes and culture types were contributing factors for observed differences in growth, physiology, and composition, resulting in characteristically unique oysters from either site.

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Chapter 1: General Introduction

To date, studies have only compared Alaskan maricultured Pacific oysters *Crassostrea gigas* (Thunberg, 1793) to those grown in traditional and established oyster growing regions but without the use of genetically identical broodstock (RaLonde 1992, Oliveira et al. 2006). There are few studies comparing performance of genetically identical broodstock between locations, and none compared in Alaska (AK). Information regarding regional differences in the growth and quality characteristics between Alaskan oysters versus oysters grown in an established growing area in Thorndyke Bay, Washington (WA) will serve as a marketing tool for Alaskan oyster growers. This information will aid in the successful development and application of tailored seed stocks to improve growout performance, and improve hatchery and growout efficiency at high latitudes (Langdon et al. 2003).

Hatcheries and broodstock development programs target improvement of specific characteristics to increase demand from growers and consumers. One desirable characteristic is yield, which is defined as the amount of growth or change in weight per growout unit, e.g. weight of family per growing chamber or plant-out area. The ultimate goal of breeding programs and hatcheries, particularly in the Pacific Northwest, has been to produce generalist oysters that are successful in a range of environments and locations (Robinson & Horton 1987, Langdon et al. 2003, Evans & Langdon 2006). The United States Department of Agriculture (USDA) funded Molluscan Broodstock Program (MBP), in conjunction with the National Oceans and Atmospheric Administration (NOAA) Sea Grant Program, have been providing support to the oyster industry in the Pacific Northwest in the form of funding and research. Alaska Sea Grant, in partnership with the MBP (Newport, Oregon (OR)), and the Alutiiq Pride Shellfish Hatchery (Seward, AK) are involved a long-term project that aims at improving yields of Pacific oysters for

Alaskan growers. Their continuing goals are to gather growth and survival data from farms and experimental growout sites using selected oysters to produce high yielding seed for the industry.

Shellfish farming in AK is expensive (Harrington 2005, RaLonde et al. 2008), and to reduce production costs Alaskan farmers have participated in the USDA MBP program since 1999 by growing selected families of Pacific oysters at growout sites in Prince William Sound and Kachemak Bay. The purpose of the MBP project has been to develop oyster broodlines that are characterized by higher yield, faster growth, and better survival. To date, substantial increases in yield, growth, and survival have been achieved. However, Alaskan oysters are sold live to the halfshell market that demands high quality meat in addition to yield. Alaskan farmers also need quantitative quality data from other regions in order to differentiate their product in the market.

Historically, oyster farming started in AK in 1909 with seed plantings on intertidal beaches from Ketchikan, in the most southern part of the state, to Kachemak Bay, 1,000 km to the northwest. Production peaked at 550 gallons (~2,100 L) of shucked meat in 1943 with the industry surviving only in the southern-most regions (Yancey 1966). Struggles with unwieldy regulations and difficulties with the remoteness of the farms eventually led to the industry's demise in 1967. The industry began again in the mid-1970s growing oysters on surface trays for the live oyster half shell market (RaLonde 1992). Passage of the Alaska Aquatic Farm Act of 1988 provided a simplified and standardized regulatory framework, which encouraged farming permit applications and industry growth until recent years, which have seen a decline in both production and sales of oysters since 2005 (Fig. 1.1A; 1.1B).

Water conditions, particularly temperature, salinity, food availability, reproductive development, and stress are well-established factors contributing to differences in growth and biochemical composition of Pacific oysters (Bernard 1983, Brown & Hartwick 1988, Quayle

1988). Environmentally influenced compositional changes in oysters and similar bivalves are partly controlled by inherent genetic predisposition (Beattie et al. 1980, Gricourt et al. 2003, Bacca et al. 2005, David et al. 2005), but are also dictated by adaptive responses (Hochachka & Somero 1973, Berthelin et al. 1990, Ruiz et al. 1992, Bougrier et al. 1998). The accumulation, storage, and utilization of biochemical resources in oysters are determined by seasonal environmental patterns of temperature and food availability (Berthelin et al. 1990, Whyte et al. 1990), which regulates reproductive development (Mann 1979). Oysters require glycogen, lipid, essential fatty acids, minerals, and protein as energetic and structural component of gametes (Walne & Mann 1975, Mann 1979, Whyte & Englar 1982, Berthelin et al. 1990, Whyte et al. 1990, Ruiz et al. 1992, Soudant et al. 1999).

The MBP has been successful to date in improving growth of Pacific oysters, but little work has been conducted to elucidate the effects of selection upon the intrinsic quality of oysters. Langdon et al. (2003) observed positive correlation in yields between oysters of the same genetic stock grown under intertidal and suspended culture conditions at sites in Northern California, OR, and WA. Results indicated that selection for high yield in one environment would likely result in a correlated response in another environment. The study called for further evaluation of families across a wider range of environments on the Pacific coast, with a view to determining whether substantial improvement of yields as well as oyster quality can be achieved by selecting for 'generalists', or whether it will be necessary to select lines which are suited for particular sites. Demand and the potential for growth of the industry in AK and the desirable benefits of the attributes listed above prompted inclusion of sites in the state to MBP experimental trials.

Using selected animals from the already successful MBP can determine whether current families are appropriate for use in the future development of hatchery and breeding programs in AK, or whether a unique broodstock is needed. Furthermore, the information generated will

provide useful quality data which will be made available to growers and buyers of Pacific oysters from the Kachemak Bay and Thorndyke Bay regions enabling them to characterize and trademark their product (Fig. 1.2).

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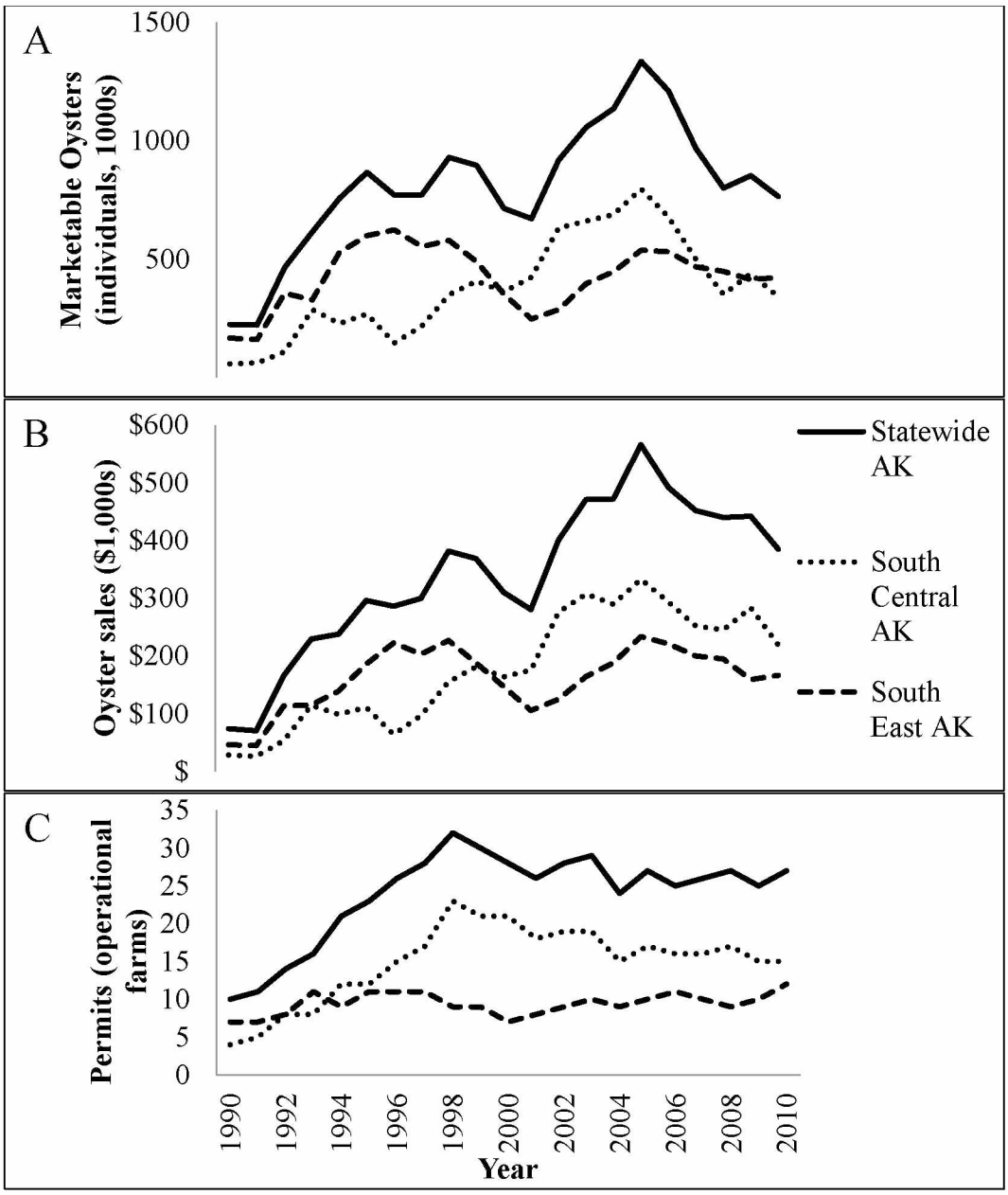

Figure 1.1. Alaska Department of Fish and Game (2011) Pacific oyster production statistics by region (1990-2010). (A) Number of marketable oysters. (B) Oyster sales. (C) Number of operating permits.

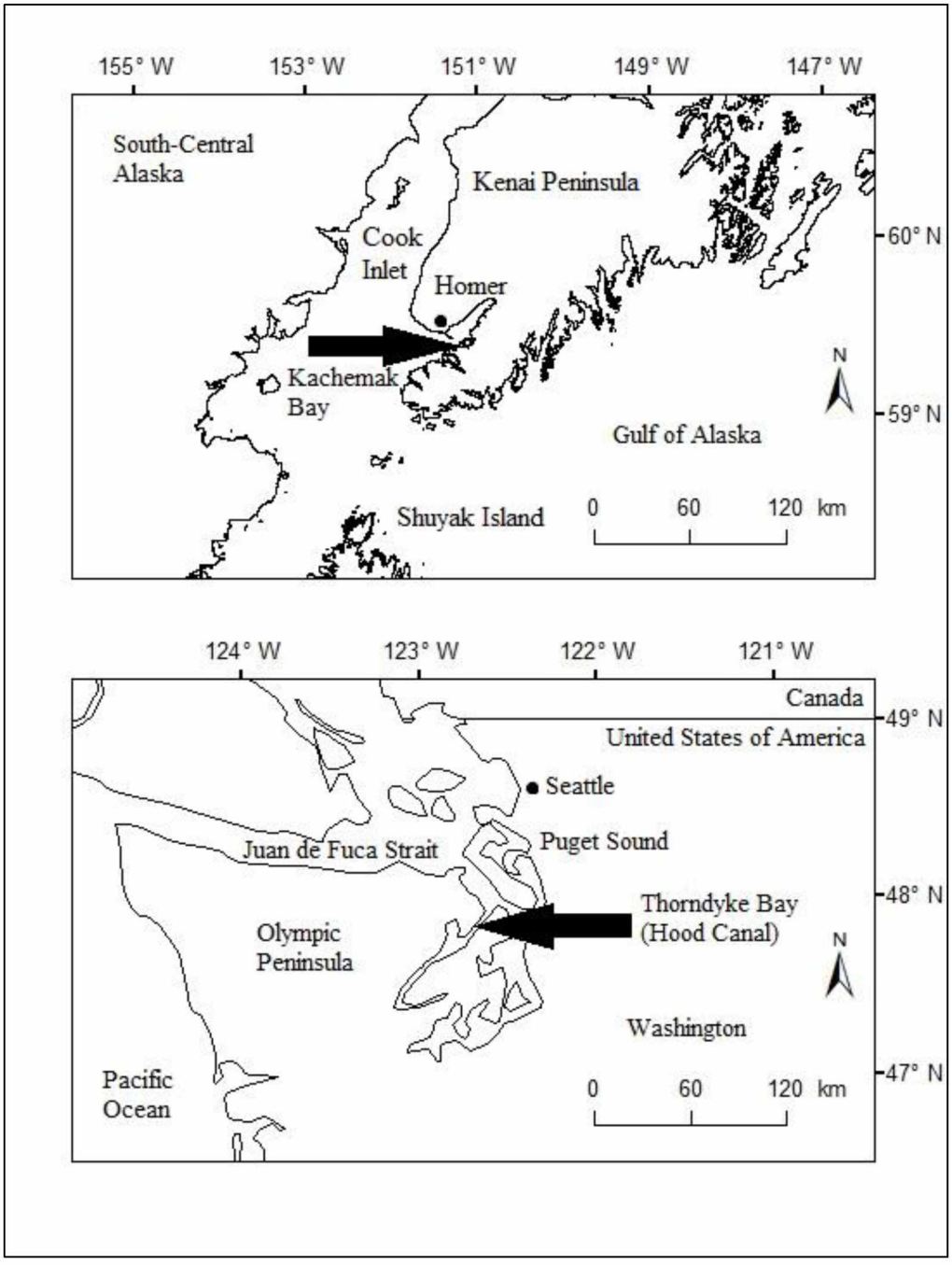

Figure 1.2. Maps of growout sites at Kachemak Bay, AK and Thorndyke Bay, WA.

Chapter 2: Growout study of selected families of Molluscan Broodstock Program oysters cultured in Kachemak Bay, Alaska, and Puget Sound, Washington[1]

2.1. Abstract

The primary objective of this project was to evaluate the growth, after three years, of families of Pacific oysters (*Crassostrea gigas*) from the USDA-funded Molluscan Broodstock Program (MBP), planted using suspended culture techniques at Kachemak Bay (KB), Alaska (AK), and at an intertidal site in Thorndyke Bay (TB), Puget Sound, Washington (WA). The MBP selects oysters to improve yields, growth, and survival, but little is known about the effects of selective breeding on other properties of selected oysters. Growth, biometric, and shell shape characteristics of oysters sampled in October of 2009 from each of the seven highest-yielding MBP families were recorded. These characteristics were compared with a control family, sourced from wild and industry stocks, at each site. Selected oysters had a higher total yield, growth, survival, shell weight, meat yield, and cavity volume in MBP Cohort 20 oysters over three years of growout when compared to a control family at KB. Oysters from KB were also slower growing and significantly smaller than at TB, and shell shape characteristics varied between culture types. Information from this study will be valuable for marketing Alaskan oysters and further development of breeding programs.

[1] Thomas, S.R., A.C.M. Oliveira, R. RaLonde, C.J. Langdon and G. Eckert. Growout study of selected families of Molluscan Broodstock Program oysters cultured in Kachemak Bay, Alaska, and Puget Sound, Washington. This chapter has been prepared for submission in the Journal of Shellfish Research.

2.2. Introduction

The Pacific oyster *Crassostrea gigas* (Thunberg, 1973) was introduced to the West Coast of the United States from semi-temperate regions of Japan in the early 1900s as a species for mariculture. Pacific oyster aquaculture is successfully established in regions of North America outside of its native range of Japan and Korea (Carrasco & Baron 2010), into Alaska (AK), British Columbia, and Washington (WA). Water temperature variability in the Pacific Northwest leads to inconsistent reproductive maturity and unreliable natural recruitment, causing oyster growers to be reliant on hatchery produced seed to augment natural recruitment to sustain harvests (Robinson & Horton 1987). Oysters do not reach full reproductive maturity in the cold water of AK, requiring farmers to be completely reliant on hatchery produced seed, increasing the cost of production for Alaskan farms (RaLonde 1992, RaLonde & Painter 1993, Conte et al. 1997, Harrington 2005). Colder conditions also depress metabolism and slow oyster growth (Hochachka & Somero 1973, Mann 1979).

In AK, oysters are farmed almost exclusively using suspended culture techniques, where lantern nets are suspended two meters below the surface by buoy or log floatation. Recently, a method of suspending wire cages from rafts has been developed as an alternate oyster growout method. Suspended culture allows continuous exposure to phytoplankton, reduces the threat from benthic predators, and prevents the possibility of freezing during winter. Suspended culture also results in unique growth characteristics. Shells can be thinner and less dense as oysters develop a different crystalline structure under suspended culture conditions, possibly due to reduced requirement for protection against abrasion and desiccation they would experience in the intertidal zone (Galtsoff 1964, Whyte & Englar 1982). In WA, oysters destined for live sales are predominantly farmed intertidally directly on the beach, or in bags and racks anchored close to the substrate. The two contrasting culture types present different conditions to the shellfish, which

lead to different growth characteristics (Whyte & Englar 1982), physiology (Bougrier et al. 1998), and survival (Rafrafi & Uglow 2009). Previous studies have investigated the effects of differing placement in the growout environment upon growth (Degremont et al. 2005), as well as the effects of suspended culture lantern net culture (Brown & Hartwick 1988), and of the effects of bottom culture upon growth characteristics (Whyte & Englar 1982, Costa Muniz et al. 1986). Other researchers have examined the effects of differing environment between growing locations upon oyster growth in the Pacific Northwest (Brown 1988, Evans & Langdon 2006).

Hatchery production and broodline development programs target growth characteristics such as improved yields, growth, survival, and reduced variability in order to improve the production and quality of oysters for growers and consumers (House et al. 2003, Harrington 2005). One desirable characteristic for growers is yield, which is defined as the change in weight per unit, growout chamber, or planted area. Yield is determined by both growth (change in mean individual weight), and survival. The Molluscan Broodstock Program (MBP) has selected generalist oyster families that are successful over a range of environments and locations (Robinson & Horton 1987, Langdon et al. 2003, Evans & Langdon 2006). The MBP has used test sites in AK previously, but in recent growout studies sites have been located in either WA or Oregon (OR).

Growing conditions in AK are very different from those in more southern regions of the Pacific Northwest. Alaska Sea Grant, in partnership with MBP and Alutiiq Pride Shellfish Hatchery in Seward, AK, is involved in a long-term project aimed at improving yields of Pacific oysters for Alaskan growers. Information on how broodlines perform in Alaskan conditions will aid in the development of region specific broodstock and ultimately benefit growers in the state. Langdon et al. (2003) observed positive correlation in yields between oysters of the same genetic stock grown under intertidal and suspended culture conditions at sites in Northern California, OR,

and WA. Results indicated that selection for high yield in one environment would likely result in a positive correlated response in another environment. The study called for further evaluation of families across a wider range of environments on the Pacific coast, with a view to determining whether substantial improvement of yields, as well as oyster quality, can be achieved by selecting for 'generalists' or whether it will be necessary to select lines which are suited for particular sites such as AK. The development of high yielding, generalist oysters enables breeding programs and hatcheries to service the needs of the many and diverse farming operations on the West Coast using groups of families as broodstock.

In this study, we investigated the effect of different environments on yield, growth, survival, biometric characteristics, and shell shape among families derived from selected MBP broodstock, in both Kachemak Bay, AK, and Puget Sound, WA. Performance of MBP families was compared to that of industry and wild stocks of oysters. The objective of the study was to provide oyster growers in AK and WA with information on the characteristics of individual oysters from their farm locations and the differing culture types typical to each region. We tested for differences in growth and physical characteristics between selected MBP families and control families, among oysters between growing environments, between suspended culture versus bottom culture, and between individual families. In AK the growth and quality information collected will aid farmers to boost production to meet the market demands for the state. The performance of selected MBP oyster families will provide a foundation and set the requirements to establish an oyster breeding program tailored for AK.

2.3. Materials and Methods

The growout sites in this study are located in areas with profoundly different climatic regimes and farming practices. In AK, oysters are grown exclusively using suspended, long-line, lantern net culture techniques; in contrast, WA oysters at Thorndyke Bay are grown on the bottom in the intertidal zone. The higher latitude of the growout site at KB (59 °34' N, 122 °16' W) has a significantly colder water temperature regime throughout the year when compared to the lower latitude of the growout site at TB (47 °81' N, 122 °66' W). Alaskan summers are short with long sunlight hours at mid-summer with water temperatures at Kachemak Bay never exceeding 20°C. The winters are long and the days short, the atmospheric and oceanic conditions are cold, and water temperature is relatively low throughout much of the winter, often reaching close to 0°C mid-winter. In WA, summer starts earlier and lasts longer, with shorter summer days but warmer water temperatures occasionally exceeding 20°C (Figure 3.2). Both Kachemak Bay (AK) and Hood Canal (WA) waters have good primary productivity suitable for oyster growth, but with slightly differing diatom and dinoflagellate abundance and bloom timing regimes (Larrance et al. 1977, Strickland 1983, Field & Walker 2003, KBRR NERRS System Wide Monitoring Program 2010, NANOOS website 2011, NERRS website 2011). Differing placement in the environment (Degremont 2005), climatic regimes and timing of food abundance and genetic background affect growth rates of Pacific oysters (Bernard 1983, Brown 1988, Brown & Hartwick 1988, Flores-Vergara et al. 2004, Evans & Langdon 2006).

2.3.1. Environmental conditions

The MBP growout site at Kachemak Bay is located in Peterson Bay, near Homer, AK. A deep sheltered inlet extending ~2 km into the Southern coast of Kachemak Bay (59°58' N, -151°28' W), Peterson Bay is ~35 km east of the entrance to the Lower Cook Inlet which opens on

to the Gulf of Alaska and circulating coastal currents. The bay lies opposite the Homer Spit which extends ~7 km into Kachemak Bay, where average bottom depth is ~30 m. The protruding Homer spit determines water conditions in the bay, as it acts as a barrier to currents and tidal drainage. Kachemak Bay is an estuarine environment, which receives oceanic nutrient-rich water from localized upwelling in central lower Cook-Inlet (Muench et al. 1978, Abookire & Piatt 2005). Periods of stratification and mixing are dependent on wind, glacial melting, and precipitation that are determined by seasonality. The National Estuarine Research Reserve System (NERRS website 2011) collaborative water quality project website (http://www.nanoos-shellfish.org/Station-Data/Alaska/Homer-Ferry-Terminal-Dock/15.aspx) describes Kachemak Bay as 'a predominately estuarine environment during summer months when glacial runoff is highest, during the winter months reverting to a more marine-like system with glacial runoff at a minimum'. Kachemak Bay has a unique oceanographic condition in the form of a 'double gyre' system, which has the effect of increasing water retention times in the outer bay (Larrance et al. 1977). The increased retention time of waters in Kachemak Bay is a dominant factor contributing to large spring and summer phytoplankton populations. The result is productive water conditions suitable for oyster aquaculture operations, of which there are a number in the local area.

The MBP growout site in WA was located at Thorndyke Bay in the Hood Canal (47 °81'N, -122 °66'W). Thorndyke Bay is a small obtusely lunar shaped bay with a mixed and sandy/muddy bottom less than 1 km wide open to the Hood Canal. Thorndyke Bay is located in the central region of the Hood Canal ~ 70 km south of the entrance to the Juan De Fuca Strait which ultimately opens into the northwest Pacific Ocean ~ 120 km to the West. Water exchange in the deep fjord like system of the Hood Canal (up to 200 m deep) is slow and retention times long due to a large sill near the mouth and relatively long distance from the ocean. Waters in Hood Canal are susceptible to low dissolved oxygen concentrations and hypoxic conditions, due

to high phytoplankton productivity in shallow waters and high levels of stratification resulting from low seawater flushing and freshwater run off (Hood Canal Dissolved Oxygen Program – HCDOP website 2011). Hood Canal is high in primary productivity. Blooms occur periodically, and are dependent on a complex relationship between freshwater runoff, circulation, upwelling at deep-water sills, wind-mixing, and solar irradiation (Strickland 1983). The waters support multiple species of successful historic, wild, and introduced commercial shellfish populations.

Surface temperature was continuously monitored at the Kachemak Bay, MBP growout site from the beginning of the growout period until May 5 of 2010 with a recorder (HOBO-TidbiT v. 2 Temp Logger, Pocasset, Massachusetts, USA) on Homer ferry dock at the end of the Spit by the NERRS local operation for Kachemak Bay. Absent data from NERRS recording was filled with temperature data recorded every 4 hours using a data logger (HOBO- Water Temp Pro v. 2 Logger, Pocasset, Massachusetts, USA) sited in Peterson Bay at the Kachemak Bay Shellfish Nursery near the growout site opposite the Homer Spit.

The NERRS website also provides typical values for salinity at Kachemak Bay based on data from monitoring equipment placed on Kachemak Bay ferry dock. The observation station was located north of the growout site on the opposite side of Kachemak Bay on the Homer Spit; observed average winter salinities at Kachemak Bay ranged from 15-30 psu (2.2- 4.4 kpa). 'Normal' summer salinities at Kachemak Bay range from 25-32 psu (3.6-4.6 kpa), but can be heavily reduced by increased glacial runoff during warm summer periods (Muench et al. 1978, Field & Walker 2003, Abookire & Piatt 2005).

Continuous water temperature monitoring did not occur at the TB site. A temperature profile was generated using data from a monitoring station in nearby Seattle, Puget Sound, WA. Hourly temperature data were taken from the National Oceans and Atmospheric Administration (NOAA), Tides and Currents Website, Meteorological Observations Station 9447130

(http://tidesandcurrents.noaa.gov/data_menu.shtml?stn=9447130%20Seattle,%20Puget%20Sound,%20WA&type=Meteorological+Observations). Daily temperature data provides a continuous temperature profile for Seattle, which showed a similar pattern to available discontinuous data to the temperatures observed at the experimental site in Thorndyke Bay (not shown).

The data from Seattle was used for comparison of water temperatures over the experimental period to compare to the nearby Thorndyke Bay site and KB (Fig. 2.2). Using 44 mean monthly temperatures from Kachemak Bay and Seattle an unpaired Student's T-test was used to compare the two regions of AK and WA, respectively, for differences (87 degrees of freedom).

2.3.2. Experimental oysters

In AK, oysters are grown almost exclusively using suspended, long-line, lantern net culture techniques. Post-harvest quality assessment examined MBP Cohort 20 grown at Kachemak Bay and Thorndyke Bay. Cohort 20 was created in April of 2006 by crossing the highest yielding families from Cohorts 11 and Cohort 16 (Fig. 2.1), resulting in 45 selected families. Four separate pairs of control families were also created. All crosses, hatchery, and nursery husbandry techniques followed methods outlined by Langdon et al. (2003). On October 23 of 2006, 35 oysters from each Cohort 20 family were stocked into each of eight 3 mm mesh scallop spat collector bags. In contrast, WA oysters are grown on the bottom in the intertidal zone. Siblings from the same MBP and control families were concurrently planted at Thorndyke Bay, employing standard bag-on-bottom culture techniques (Langdon et al. 2003) with a stocking density of 50 oysters per bag. Fouling removal occurred at both locations in the spring and fall of 2007 using high-pressure water treatment at approximately 50 psi (345 kpa).

Initially, four separate pairs of control families were created to serve as "unselected" control families by cross-breeding oysters from "wild" populations, oysters from sources within the industry, and from "standard" MBP broodstock. Each pair of control families was created from crosses of two broodstock families. Each control family was split and stocked into two replicated growout chambers at both experimental sites.

Control families 65 and 66 were created from crosses of Dabob Bay wild oysters. Dabob Bay is located in the Hood Canal, WA (47°83' N, 122°81' W) very close to TB (47 °81' N, 122 °66' W). The tideland is dominated by a sand substrate with spots of mud. It should be noted that the genetic profile of oysters categorized as 'wild' in WA are highly likely to be contaminated from cultured stock grown in nearby culture areas. Families 67 and 68 were wild crosses sourced from Taylor Shellfish Grower's culture site at Willapa Bay. Willapa Bay is located in southern WA (46°55' N, 124°0' W). It is an inlet sheltered by a large land spit. Oysters cultured in Willapa Bay are grown in areas of sandy substrate, or on silty, muddy bottom. Families 69 and 70 were created from crosses of Coast Seafood Company oysters between female oysters from Humboldt Bay, CA and male oysters from Dabob Bay, WA. Humboldt Bay is located in Northern California (40°74' N, 124°22' W). It is bar created lagoon, composed of clayey intertidal silts and sandy substrate. "Standard" control families 71 and 72 were created by crossing families formerly identified as average performers from MBP broodstock. These were Cohort 16 family 35 and Cohort 7 family 51, from the HMSC, OR.

Heavy losses at both KB and TB during the growout period resulted in the need to combine the four initially planned control families to form one group. Only families 65, 66 and 70 remained with sufficient numbers to meet minimum sample size for the intended analyses. Families 65, 66 and 70 were pooled to create one control group, designated: Family 0.

2.3.3. Field evaluation, oyster sampling, and post-harvest assessment

October was chosen for fall sampling because it was a period of both biological and environmental similarity between the two sites. Growth conditions in October were similar at both sites: water temperatures were likely below 10.5°C, which is the lower threshold for reproductive development, after which, any developed gametes should have been re-adsorbed into the body tissues (Mann 1979; Fig 2.2). October is also a period associated with late summer primary productivity at both Kachemak Bay and Thorndyke Bay (Personal communication: Ray RaLonde 2011; unreferenced); at this time oysters were feeding and subsequently assimilating more food to energy storage and growth after a period of repose (Mann 1979, Whyte & Englar 1982). As a result oysters are typically in ideal condition for harvest, sale, and consumption in October, and therefore was a suitable time to study growth characteristics prior to winter.

At Kachemak Bay, slow growing conditions linked to lower than decadal average temperatures led to the delay of the initial planned transfer of oyster seed from small mesh bags to lantern nets in 2007 (Personal communication: Ray RaLonde 2009; unreferenced). Instead, seed was sorted and dead and non-growth animals were discarded between May 22 and 23 of 2008. The remaining selected and control families of oysters at Kachemak Bay were counted and transferred into individually labeled 18 mm mesh size lantern net compartments. At this time the surviving oyster seed in the corresponding families at Thorndyke Bay were also transferred from 3 mm small-mesh scallop spat bags to 6 mm mesh growout bags (Norplex Inc., Auburn, Washington, USA).

Mean family survival, family yield (mean live weight per compartment), and individual final weight (g individual^{-1}) data were collected at the first sampling in October of 2009. From these collected data, the average individual oyster weight per chamber was calculated by dividing total compartment weight by the number of live oysters (g individual^{-1}). Survival data was

calculated as the percentage of animals alive at sampling in October of 2009 as a proportion of the numbers of oysters present at initial planting at the experimental sites at Kachemak Bay (35 individuals compartment^{-1}), and Thorndkye Bay (50 individuals compartment^{-1}). Four replicates were created within a family. Duplicates were created within the four replicates identified by four color-coded blocks, each consisting of two chambers each within a family grown in randomly distributed chambers or bags. Average values were calculated for survival, yield, and individual weight values per family using the replicate groups.

Oysters were sampled from suspended lantern nets at Kachemak Bay on October 6 and 7 of 2009, and from the intertidal bags at Thorndyke Bay on October 21 of 2009. At Kachemak Bay, oysters from all 45 of the MBP Cohort 20 families and the control families were weighed and counted to determine yields, individual wet weights, and survival values. Families were ranked based on yield (mean weight of surviving oysters per container) and the top seven yielding families were identified. The top-performing AK families were retained at the Kachemak Bay and Thorndyke Bay site to allow comparisons of the same families between the two sites. The top seven AK families at each site were cleaned of biotic and abiotic fouling and sampled, together with the control family. The remaining unused families were donated to the farm operators at each site once high performance oysters were identified and harvested for subsequent testing.

For post-harvest analysis of individual oysters three oysters per color-coded block within a family were randomly sampled, which resulted in 12 animals from each of the seven families and the control family. This resulted in 96 oysters sampled per sampling period from each site. Remaining oysters from the top seven yielding families and control family ($\geq$ 24 animals per family) sampled in October of 2009 were returned to the lantern nets and placed back at growout site for a later analysis, which included a spring sampling in order to conduct a seasonal comparison of quality (see Chapter 3).

Samples of individual oysters from a site were placed in wet-lock boxes with cold gel packs for transportation by air to the University of Alaska (UAF) Fisheries Industrial Technology Center (FITC) in Kodiak, AK.

2.3.4. Oyster biometrics and shell shape

At each site, 12 oysters (three randomly chosen from each of four replicate bags) were collected from each high performance family, then weighed and measured. Individual oyster measurements of shell length (anterior-posterior), shell width (dorsal-ventral at the widest point), and cup depths were taken using digital Vernier calipers with a 200mm range (Fowler and NSK, Newton, Massachusetts, USA). Individual oyster weights were measured using a top loading scale with manufacturer accuracy of 0.01g (Mettler Toledo, Columbus, Ohio, USA).

In order to assess oyster shape and marketability, oyster researchers standardize shell dimension measurements of length, width, and cup depth. Measurements were used to compute shell shape index, a non-invasive index developed by Imai & Sakai (1961) determines the relative cup depth of the oysters and their economic value using shell dimensions and is often referred to as the economic condition index (CI^E).

$$CI^E = shell\ cup\ depth\ \times\ [0.5\ (shell\ length + shell\ width)]^{-1}$$

Equation 2.1

Imai & Sakai (1961).

A second and third index use shell dimension data to separately calculate ratios of oyster shell width and cup depth to shell length.

$$CI^{(B)}\ D/L\ =\ shell\ cup\ depth\ /\ shell\ length$$

Equation 2.2

&

$$\mathrm{CI}^{(\mathrm{B})}\ \mathrm{W/L} = shell\ width\ /\ shell\ length.$$

Equation 2.3

Brake et al. (2003).

2.3.5. Data analysis

Data was summarized for each family at each site as the mean and standard deviations determined from replicate measurements for all parameters. Percentage data (y) were transformed using an Arcsine transformation in order to centralize the data about the mean, as follows:

$$Sin^{-1}\ (\sqrt{y})$$

All statistical tests were performed using Statistica v. 9.0 (Statsoft, Tulsa, California, USA). In order to conduct reliable Analysis of Variance (ANOVA), data were tested for normality using a Shapiro-Wilks test (Shapiro-Wilks; 95% confidence; $P < 0.05$). All growth and biometric data were normally distributed.

In order to eliminate the bias of deliberate selection of the top seven yielding families, parameter values were standardized to show standard deviations about the collective mean of all families including the control family. Matching data of an individual parameter was standardized to create a z-score (Equation 2.4). The z-scores were calculated for individual families using difference of the mean (x) of the parameter within a family to the mean of all families ($\bar{x}$) divided by the standard deviation of the mean (σ). Calculated z-scores for parameters of yield, growth, and survival of families were plotted against one another within sites, where z was estimated as:

$$z = \frac{x - \bar{x}}{\sigma}$$

Equation 2.4

The z-score allowed for comparison of family yield, growth, and survival to the mean of the identified top seven yielding and control families. Regression analysis between sites for yield, growth, and survival generated R^2 values (Significant: $P < 0.05$) to test for correlation in parameters between sites.

Yield, growth, survival, biometric, and shell shape data among oysters of the top seven yielding MBP families and the control family were compared for differences using a Tukey's Honest Significant Differences Test (ANOVA: Post-Hoc). Growth and morphometrics of oysters grown at Kachemak Bay in AK were compared with those of oysters grown at Thorndyke Bay. Yield, growth, survival and biometric data was compared between sites and between the mean of all MBP families versus control families using ANOVA ($P < 0.05$) and linear regression of z-scores.

2.4. Results

2.4.1. Environmental conditions

At Kachemak Bay (KB), the mean temperature between January of 2007 and May of 2010 was 6.2 ± 3.1°C, the maximum temperature was 12.2°C on July 3 of 2009, and the minimum temperature of 0.5°C was reached on March 15 of 2007. At the Seattle observation station, the mean annual temperature between January of 2007 and May of 2010 was 10.4 ± 1.8°C, the maximum temperature was 13.9°C reached July 4 of 2009, and the minimum of 7.5°C reached on March 19 of 2008. Temperature monitoring data available from the NANOOS website (2011), from nearby Dabob Bay in the Hood Canal, observed surface temperatures as high as 20°C in mid to late June and repeated in the summers of 2007 to 2009.

Mean annual temperatures in the temperate region of Puget Sound, WA were significantly greater than at KB, AK, in each year from 2006 to 2009 (ANOVA: $P < 0.05$; Fig.

2.2). The mean temperature for the entire period 2006-2010 was also significantly higher in WA than in AK ($P < 0.05$). It is important to consider that the WA oysters growing in the intertidal zone experience the effects of ambient air temperatures during the twice-daily exposure of low tide, but this study did not monitor these micro-scale environmental factors.

2.4.2. Field evaluation and post-harvest assessment of the top seven MBP family lines – KB families

The top seven yielding families as identified at the suspended culture site at KB on October 6 of 2009 are presented with the corresponding families sampled from the intertidal site from TB on October 21 2009 (Table 2.2 and Fig. 2.3).

The mean family yield for all families, including the control, at KB (35 animals chamber^{-1}) was multiplied by a factor of 1.43 in order for values to match a chamber density of 50 animals as at TB. Mean yield at KB and TB was 1,022.9 ± 365.8 g chamber^{-1} and 5,322.4 ± 1,338.0 g chamber^{-1}, respectively. There was no relationship in mean family yields between sites at the end of the growout period ($R^2 = 0.08$, $P > 0.05$; Fig. 2.4A). In October of 2009, mean family yield was significantly greater (ANOVA, $P < 0.05$) among selected families of MBP oysters when compared to control families of oysters at KB (Tables 2.2 and 2.3). At TB there were no significant differences detected (ANOVA, $P > 0.05$) in mean family yield between selected and the control families of oysters (Tables 2.2 and 2.3). Mean family yields in each growing chamber were significantly lower at KB than at TB despite standardizing values to account for differences in stocking density (ANOVA, $P < 0.05$; Tables 2.2 and 2.3).

At KB, mean individual oyster growth per family was 37.9 ± 7.1 g individual^{-1}, and 182.4 ± 18.5 g individual^{-1} at TB. There was a positive correlation in growth of MBP families between KB and TB ($R^2 = 0.71$, $P > 0.05$; Fig. 2.4B). In October of 2009, mean oyster growth was

significantly greater (ANOVA, $P < 0.05$) among selected families of MBP oysters when compared to control families of oysters at KB (Tables 2.2 and 2.3). At TB, there were no significant differences detected (ANOVA, $P > 0.05$) in mean oyster growth between selected and control families of oysters (Tables 2.2 and 2.3). As with mean yield, the mean oyster growth in each growing chamber was significantly lower at KB than at TB (ANOVA, $P < 0.05$; Tables 2.2 and 2.3).

Mean survival per chamber at KB and TB was 52.4 ± 17.0 % and 59.2 ± 15.0 %, respectively (Table 2.2). Survival among MBP families showed a significant negative correlation between KB and TB in October 2009 ($R^2 = 0.60$, $P < 0.05$; Fig. 2.4C). As with yield and growth, mean survival per chamber was significantly greater (ANOVA, $P < 0.05$) among selected families of MBP oysters when compared to control families of oysters at KB (Tables 2.2 and 2.3). But at TB there were no significant differences detected (ANOVA, $P > 0.05$) in mean survival between selected and control families of oysters (Tables 2.2 and 2.3). A comparison of survival between locations indicated that mean survival of all oysters was significantly lower at KB than at TB (ANOVA, $P < 0.05$; Tables 2.2 and 2.3).

Yield correlated positively with survival at KB ($R^2 = 0.92$ $P < 0.05$, Fig. 2.3), while at TB yield and survival had a positive yet non-significant correlation ($R^2 = 0.43$, $P > 0.05$; Fig. 2.3). Conversely, mean family yield and survival was similar between selected families of MBP oysters and control families at TB (ANOVA, $P > 0.05$; Tables 2.2 and 2.3).

2.4.3. Oyster biometrics and shell shape

At the end of the growout study, mean whole weight of individual oysters grown at KB was 45.1 ± 4.0 g, and 194.4 ± 13.1 g at TB (Table 2.4). Mean shell weights at KB were 31.5 ± 2.6 g, and 132.4 ± 9.2 g at TB. At KB, mean whole weights and shell weights among selected MBP

families of oysters were significantly higher than those of the control family ($P < 0.05$; Tables 2.4 and 2.5). At TB however, no differences were detected in whole weights or shell weights of selected MBP oysters when compared with the control family ($P > 0.05$; Tables 2.4 and 2.5). Mean shell lengths at KB were 88.1 ± 4.0 mm, and 131.3 ± 5.0 mm at TB, and mean shell widths at KB were 67.4 ± 3.9 mm, and 78.3 ± 2.8 mm at TB. Mean cup depths at KB were 26.1 ± 1.7 mm, and 40.2 ± 2.7 mm at TB. There were no significant differences detected in shell lengths, shell widths, and cup depths between selected MBP oysters and the control family of oysters at either KB or TB ($P > 0.05$; Table 2.4 and 2.5). All biometric parameters were significantly lower at KB than at the TB site ($P < 0.05$; Table 2.4 and 2.5). Selected MBP oyster families had similar width/length and depth/length ratios to the control oysters at both KB and TB at the end of the growout period ($P > 0.05$, Table 2.5). The width/length ratio of oyster shells was significantly greater at KB than at TB ($P > 0.05$, Table 2.5), but depth/length ratios were similar between sites.

In October of 2009 the mean CI^E and Brake et al. (2003) width/length and depth/length ratios of oyster shells were similar between the control family of oysters and the selected MBP families at both KB and TB ($P > 0.05$). There were no significant differences detected in CI^E among individual MBP families within a site at KB or TB (Table 2.5). Family 44 had a greater CI^B W/L ($P < 0.05$) than families 21 and 24 at KB. There were no significant differences detected in CI^B D/L among individual MBP families at KB at TB; however, family 21 had a higher CI^B D/L ($P < 0.05$) than family 20 at TB.

2.5. Discussion

Evans & Langdon (2006) determined that growing environment and culture type have a significant effect on yield, individual growth, and survival, but that families from one environment may not perform well in other different environments. Evans & Langdon (2006)

called for further evaluation of families across a wider range of environments on the Pacific coast, with a view to determining whether 'generalists' established as successful in WA and OR in recent growout studies would be successful in AK, or whether specific lines which are suited for conditions of more northern latitudes need development. Our study was conducted to help answer this question, with a specific goal of assessing and developing improved broodstock to enhance oyster production in AK. We found that the MBP successfully created generalist Pacific oysters with increased yields and survival among families using selected broodstock from WA, AK, and OR.

2.5.1. Environmental conditions

Average water conditions were significantly cooler at KB than Puget Sound waters in Seattle, WA, our surrogate for conditions at TB ($P < 0.05$; Table 2.1 and Fig. 2.2). At KB, the oysters grown in lantern nets were constantly submerged and subject to the extreme low water temperatures in the winter (Fig. 2.2), a factor known to affect metabolic processes (Bougrier et al. 1998, Rafrafi & Uglow 2009), inhibit shell growth (Hochachka & Somero 1973, Vermeij 1978), and reduce feeding rates (Elsey 1933, Galtsoff 1964, Whyte et al. 1990). During the summer, melting of local glaciers at KB increased the freshwater contribution to the bay, reducing salinity (25-32 psu: NANOOS website 2011) and temperature, which ultimately decreased growth (Bernard 1983, Brown & Hartwick, 1988 Quayle 1988). At TB, oysters growing in the intertidal zone were exposed to emersion twice daily at low tide, subjecting the oysters to atmospheric and solar temperature effects (Davenport & Wong 1992), and anaerobic conditions (Bougrier et al. 1998, Rafrafi & Uglow 2009) which can all alter metabolic rates within oyster tissues (Hochachka & Somero 1973, De Zwaan 1977, Livingstone & Clarke 1983). Relatively higher

water temperatures, earlier onset of spring (Fig. 2.2) and longer periods of primary productivity, which support growth, likely counteract the negative effects of periodic emersion.

The annual mean water temperatures at KB during the experimental growout period were cooler than the decadal average (Personal communication: Ray RaLonde 2009) which led to the growout period ending a year later than scheduled to allow the oysters at KB time to grow to a marketable size. Optimal temperatures for oyster growth are between 20° to 30°C (Martin et al. 2000). Oysters grown at the higher latitudes of KB are slower growing than those grown at lower latitudes such as at TB, resulting in oysters reaching market size in 18-36 months (RaLonde & Painter 1993, Oliveira et al. 2006).

At KB, June is typically the beginning of the spring/summer diatom bloom with a second diatom bloom commonly occurring in October (Kachemak Bay Research Reserve [KBRR], System Wide Monitoring Program, NERRS, 2010). The nutritional quality of the oysters' plankton diet is an important factor influencing their growth and survival (Whyte et al. 1990, Soudant et al. 1999, Flores-Vergera 2004); for example, diatoms in particular are a highly nutritious food source for Pacific oysters relative to other species (Whyte et al. 1990, Zieman etal. 1990, Soudant et al. 1999, Flores-Vergera et al. 2004, Cooney 2005, RaLonde et al. 2008).

The growout sites at KB and TB are in bio geographically distinct regions, which significantly affects the adaptive environmental responses animals undergo in growth, metabolism, and biochemistry (Hochachka & Somero 1973, Vermeij 1978), particularly with regards to the different temperature regimes.

2.5.2. Field evaluation and post-harvest assessment

Langdon et al. (2003), observed weak positive correlation in yields between oysters of the same family grown using inter-tidal and suspended culture techniques at different sites,

indicating that selection for high yield in one environment would likely result in a low correlated response in another environment, although their study did not take place across such a steep environmental gradient as in this study. The MBP Cohort 20 oysters used in this study were developed from broodstock pedigree, which was planted and grown in AK, WA, and OR before being artificially spawned at HMSC (Fig. 1). However, the selection and development of the most recent predecessors to Cohort 20 focused on the creation of oyster broodstock, which could be classified as 'generalist' performers for growout in WA and OR. In the case of oysters grown at KB, preferential selection of the top seven MBP broodlines showed greater yields, survival, and growth when compared to the control family (Tables 2.2 and 2.3). Selection has increased growth in *Ostrea edulis* grown in Europe and the East Coast of the USA and *Saccostrea glomerata* grown in Australia (Newkirk & Hayley 1983, Nell et al. 1996, 1999). It has also improved survival by increasing parasite resistance (Haskins & Evans 1988), as well as enhancing thermal tolerance of oysters in the Puget Sound in successive generations of *Crassostrea gigas* (Hershberger et al. 1984). Unlike at KB, the corresponding top seven selected families of oysters from MBP Cohort 20 grown at TB did not show significant improvements in yield, growth, or survival when compared to the control family (Tables 2.2 and 2.3). Furthermore, families did not show similar ranking patterns of yield or survival in KB and TB (Table 2.2). Control families were created from stocks of "wild" or "industry" raised oysters which have acclimatized in the environments of WA and OR for multiple generations. As such, it could be suggested that these oysters are "native" to these areas, certainly relative to any other growing areas. As expected when the control oysters were grown outside of their native growing area in Alaska, they were observed to be outperformed by the top seven families of MBP oysters deliberately selected at KB for vigor in terms of yield, growth, and survival. When comparing individual families, regression analysis showed that MBP families exhibiting good family yield and survival when

grown in KB do not necessarily exhibit good family yield and survival amongst MBP and control oysters grown at TB.

Identifying the top seven yielding families at KB meant that we were able to establish which MBP families would perform well when grown under Alaskan conditions. As expected, the same families that performed well in terms of yield performed well in terms of survival, as more surviving oysters over the growout period leads to more animals contributing to final yield. However, the lack of correlation and different rank in yield and survival of MBP families between sites suggests that oysters which have been established as 'generalists', performing well in the growing regions of both WA and OR, are not the same families which do best when grown under conditions at KB in AK. Individual oyster growth had a significant positive correlation between families at KB and TB, with five MBP families showing z-scores above the mean for both locations (Fig 2.4B). This suggests that an MBP family selected for growth will perform well in environments such as KB or TB, even when grown using different culture techniques. Nonetheless, to accurately examine the effects of selection it is necessary to conduct analysis using a greater number of families. Using the growth characteristics of all families in the cohort, performance of selected and control families can be assessed against the mean of the whole collection of selected families produced in Cohort 20 (n = 45 families).

In terms of individual family performance, the comparison of z-scores is useful and enables identification of generalists. A family found in the top right quadrant, with positive z-values at both KB and TB, represents a 'generalist' family that performed above the mean at both sites. In terms of mean family yield (g chamber^{-1}), none of the selected families grown at the AK site were categorized as 'generalist' (Fig. 2.4A). In terms of individual oyster growth (g individual^{-1}), families 20, 21, 28, 34, and 46 were found in the 'generalist' category (Fig. 2.4B). With regards to survival, oysters in family 44 performed well at both sites, hence were

categorized as 'generalist'. The implication to the objective of this study is that although a number of MBP Cohort 20 families performed well when grown using suspended culture at KB, and the lack of correlated relationship to 'generalists' from WA means that there is a need for entirely unique and tailored experimental breeding program in AK. There is a case for including in the AK breeding program consistently high performers such as 28 and 46 (Fig. 2.4A-C).

High variability in growth and growth rates among broodlines may increase the frequency of sorting, reduce labor needs, and causes problems in forecasting future harvest for oyster growers. The variability that we observed between growth and dimensions of sampled oysters (Tables 2.2 and 2.4) was heavily influenced by localized variation among replicate bags or lantern net chambers, and is likely largely influenced by localized environmental factors. This is because intertidal culture results in a more heterogeneous environment for growth than suspended culture, causing more variability among replicate growout containers. Any targeted reduction in variation of growth or dimensions among MBP families may have been masked by external influences such as mechanical activity, contact with abrasive substrate material, and environmental temperature.

2.5.3. Oyster biometrics and shell shape

Oysters at KB were significantly smaller and exhibited lower weights than at TB. Cooler water temperatures can inhibit shell growth as a function of carbonate solubility and calcium availability (Hochachka & Somero 1972, Vermeij 1978), and can also reduce feeding rates (Elsey 1933, Galtsoff 1964, Whyte et al. 1990). However, the production of smaller oysters is not an issue in the marketplace because preference for oysters of varying size classes differs with consumer demographics. Vendors of oysters destined for the half shell market, as in AK, classify oysters not by weight but by size based simply on length measurements, with categories ranging

from extra small (50.8-76.2 mm) to large and above (≥127 mm) (RaLonde & Painter 1993, Harrington 2005). Although the size distribution of KB oysters is significantly smaller than those at TB (Fig. 2.5), these have a valued position in the market because many consumers of half-shell oysters prefer extra-small and small size oysters (Harrington 2005). Oysters from KB will not be competing against medium size oysters from sites such as TB, and oysters are often trademarked by the characteristics unique to that location (Brake et al. 2003).

There was a significant difference between growth and growout time of oysters at the two sites. Oysters at KB are slower growing, accounting for the discrepancy in size. Oysters from TB are almost four times heavier and larger in terms of shell dimensions than oysters from KB (Table 2.4). The implication is that oyster growers in AK can only produce marketable oysters in two to three years, whereas at TB oyster growers may be able to harvest twice in a similar timescale. Slow oyster growth rate in AK means the growers will have lower production volume (House et al. 2003, Harrington 2005).

Oyster shape is an important product attribute (Kahn & Wansink 2004). Buyers and consumers generally favor deep-cupped oysters over flat-shaped shells (Galtsoff 1964, Brake et al. 2003, Harrington 2005, Xiong et al. 2010). Farmers use a practice called tumbling, which breaks the fringe of the oyster shell, stimulating repair by the mantle. The higher the frequency of fringe breakage, the less growth effort the oyster directs into increasing length. This accumulates shell vertically at a greater rate, creating a deeper-cupped oyster. MBP oysters' farmed using suspended culture at KB received infrequent handling and no tumbling. To compensate for limited handling and no tumbling, stocking densities at KB were deliberately low to reduce the amount of contact shells could make with one another. This ensured that natural shell growth could be observed but avoided oysters shells growing into each other and fusing. In the intertidal environment, mechanical activity from wave and ocean current action naturally tumbles the

oysters causing abrasion of the oyster shell fringes. This leads to deeper cup formation and a more desirably shaped oysters as is reflected in the higher values of CI^E observed at TB compared to KB (Table 2.4).

The lack of significant difference observed in CI^E between the control family of oysters and selected MBP oysters at TB in October of 2009 suggests that shell shape is controlled by external environmental influences rather than genetic control. Although, a study including the 45 families of MBP Cohort 20 would need to be conducted to determine possible interactions between oyster genotype and environmental conditions. Oysters manifest growth when temperatures are higher and feeding conditions are good (Walne & Mann 1975, Mann 1979, Brown & Hartwick 1988, Quayle, 1988); high latitudes and low temperatures decrease soluble calcium carbonate availability (Vermeij 1978, Doney et al. 2009) and depress metabolic rates (Hochachka & Somero 1973). Lower than decadal average temperatures and significantly cooler water temperatures in AK compared to WA (Fig. 2.2) are reflected in the lack of significant difference observed in CI^E between KB and TB in October of 2009 (Table 2.5).

The MBP Cohort 20 families 44 and 28 at KB ranked consistently and statistically higher with regards to the shell shape indices CI^E and CI^B at KB at the end of the growout period in October of 2009. Conversely, families 20 and 21 were consistently lowest of the top seven highest yielding families (Table 2.5). There is little apparent change in standard deviations in the shape indices describe above between families, although error is likely masked by the precision of the presented results as these indices are in low numbers. The shell shape index (CI^E), developed by Imai & Sakai (1961) considers cup depth relative to both shell width and length. Mean CI^E at KB in October of 2009 was exactly the same as observed by Oliveira et al. (2006) at the same site in KB in October 2003 (Table 2.4).

Less handling potentially accounts for the higher values of length to width ratio in shells than reported in other studies (Brown & Hartwick 1988, Damar et al. 2005). There were no observed differences between sites or among families in the depth: length ratios of oysters. This suggests that oysters were accumulating shell length along these two axes at a proportional rate at both KB and TB. Values for the CI^B D/L and W/L matched well with those observed by Quayle (1988) in British Columbia (0.41 and 0.68, D/L and W/L, respectively). Values of D/L and W/L ratios observed in our study (Tables 2.4 and 2.5) show less variation (< 0.04) than values observed in other studies of shell shape in British Columbia (Quayle 1988), and WA and OR (Brake et al. 2003), where variation is typically between 0.05-0.1. Natural populations of bivalves can exhibit very distinct differences in shell growth rates and shape influenced by the biogeography of the region or location they are grown in (Vermeij 1978). The ratios in this study comply with "good" ratios observed by Brake et al. (2003), used to describe oyster shell shape for industry according to simple descriptive methods.

Suspended cultured oyster shells from AK tend to be thinner and more fragile (Oliveira et al. 2006, Damar et al. 2005) compared to oysters raised on or near the bottom (Whyte & Englar 1982). Oysters deposit layers of calcite-ostracum to form shells, which are frequently interrupted by more porous layers of "chalky" calcite crystals (Galtsoff 1964). Oysters grown off the bottom deposit more porous, soft "chalky layers" compared with oysters planted on the substrate (Whyte & Englar 1982). The resulting differences in whole weight and shell weight in selected MBP oysters observed at TB versus KB (Table 2.5) were a function of different growth rates between sites. Whyte & Englar (1982) proposed that oysters submerged in or near the substrate in the intertidal zone generate thicker shells for protection against abrasion and desiccation. Oysters from TB in this study were observed to have heavier and possibly denser shells than those at KB (Tables 2.4 and 2.5). When shell weight is determined as a proportion of the whole weight, KB

(69.8%) mean values are actually higher than TB values (68.1%), although shell density was not directly determined using the Archimedes principle as demonstrated by Damar et al. (2005).

2.6. Conclusions

Different culture types and environmental conditions likely influenced yield and other growth parameters at the AK and WA sites. There is evidence of strong genetic-environment effects influencing the performance of MBP families in this study, although a full comparison of all families of MBP Cohort 20 is necessary to better determine this effect. However, negative correlations of yield and survival of the KB-selected MBP families between the AK and the WA sites suggest that AK needs a specifically tailored breeding program to generate seed that would thrive under the unique conditions found at higher latitudes. Top-yielding KB families from MBP Cohort 20 should be considered for inclusion in such a breeding program.

Shell shape, such as cup depth, is affected by environmental factors such as the mechanical action of handling and wave disturbance. Suspended oyster aquaculture, common in AK, exerts limited mechanical action on oysters. Farmers need to manually 'tumble' oysters in order to break the shell fringes to promote deeper cupped oysters, which are more desirable (Brake et al. 2003). The D/L ratio at each site was similar, suggesting that the oysters grow proportionally along these axes, but that more growth happens along the horizontal plane particularly when fringes are not broken.

Oysters from AK were slower growing and took longer to reach marketable size. As a result, farmers in the region will need to rely upon high quality and consistent production in order to be competitive in the market place. Production of small, high quality oysters for the half shell trade appears to offer a market opportunity for the Alaskan oyster industry. In AK, challenging logistics, high production and transportation costs, labor intensive farming methods, and

challenging seed growing conditions highlight the need for an effective oyster hatchery seed supply produced within the state. Alaska operates one hatchery in Seward, but Alaskan oyster growers have increasingly purchased seed from out of state, citing problems with obtaining consistent seed supply, growth performance, and price. In conclusion, it is important that Alaskan hatcheries develop the capacity to supply high performance seed to Alaskan oyster growers.

2.7. References

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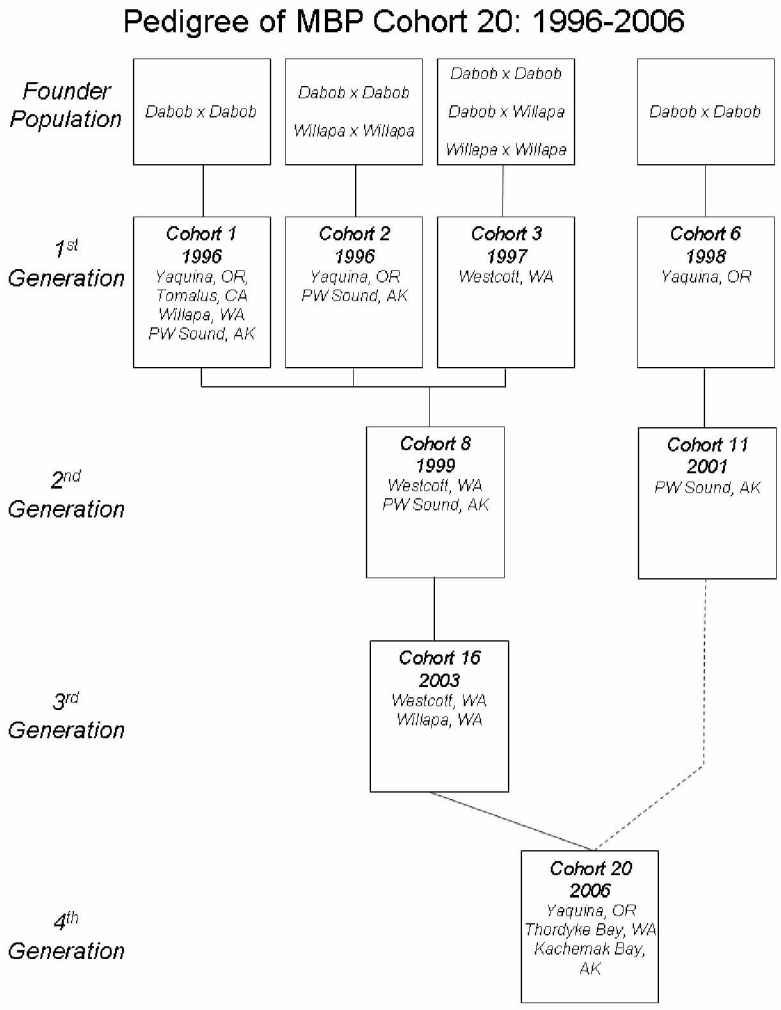

Figure 2.1. Pedigree of MBP Cohort 20 families currently being evaluated in KB, AK, and TB, WA.

Table 2.1. Min, max, and mean temperature data by year for Peterson Bay, KB, AK, (Source: NERRS Kachemak Bay and NOAA Tides and Currents, Seattle, WA (SEA), Meteorological Conditions website, daily temperature records) 2005-2010.

	Jan 2006 -May 2010		2007		2008		2009		Jan 2010 - May 2010	
	KB	**SEA**	**KB**	**SEA**	**KB**	**SEA**	**KB**	**SEA**	**KB**	**SEA**
Yearly min (°C)	0.5	7.9	0.5	7.9	0.6	7.5	0.7	7.5	0.9	8.8
Yearly max (°C)	12.7	13.6	11.9	13.4	10.6	13.6	12.7	13.9	9	NA
Daily mean (°C)	6.2	10.4	6.3	10.5	6.1	10.2	6.3	10.5	3.9	NA
± SD (°C)	(3.1)	(1.8)	(3.5)	(1.7)	(3.0)	(1.8)	(3.3)	(2.0)	(1.4)	NA

Standard deviation of the mean (SD). Data not available (NA).

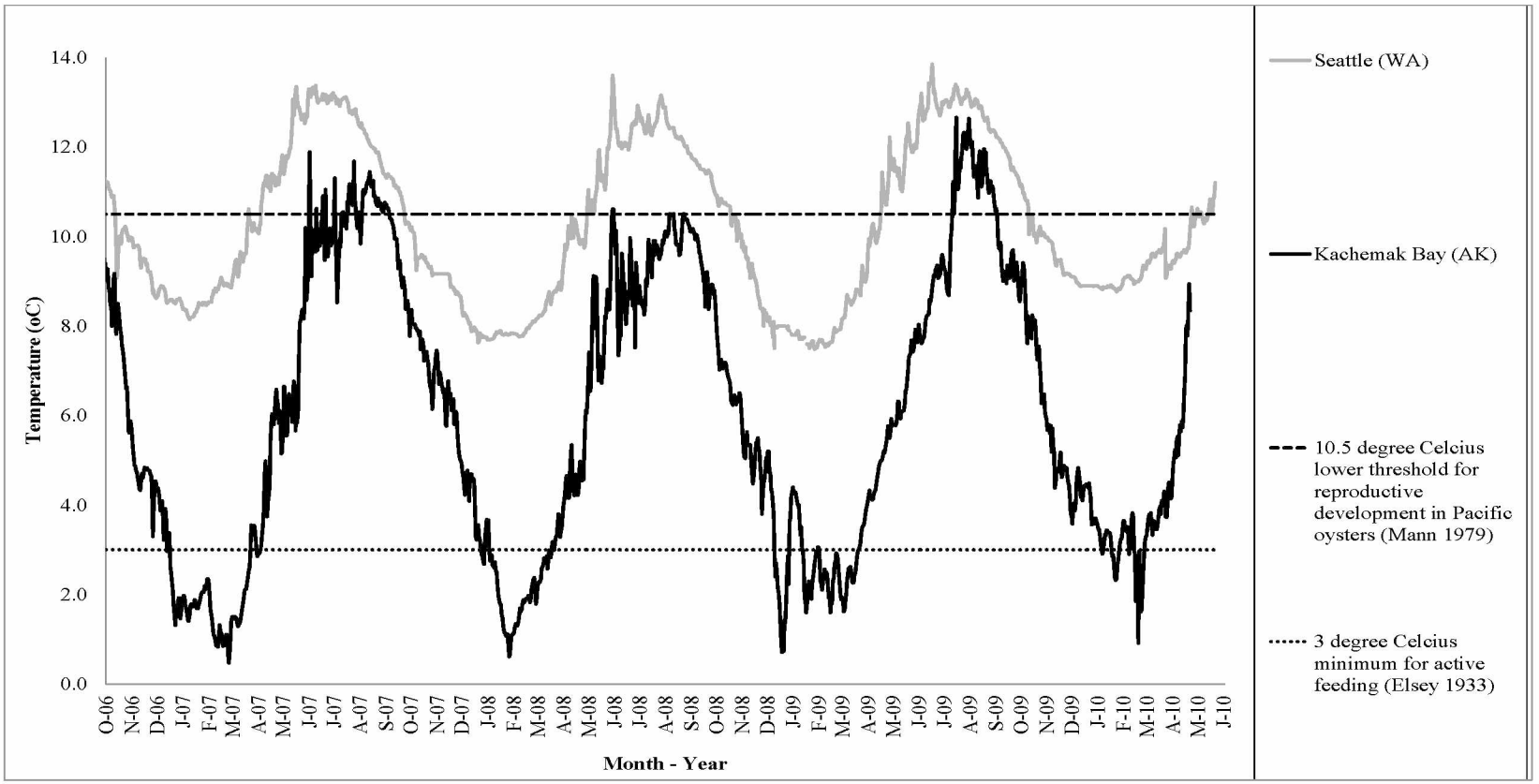

Figure 2.2. Seasonal variations in daily temperature (^{o}C) at Kachemak Bay, AK. Peterson Bay, KB, AK, oyster growout site (Source: NERRS Kachemak Bay, daily temperature records) and Seattle, WA, (Source: NOAA Tides and Currents, Seattle Meteorological Conditions website, daily temperature records), 2005 to 2010.

Table 2.2. Mean family yield, individual weight, and survival of MBP oysters at KB, AK and TB, WA from October of 2006 to October of 2009.

Family #	28	24	46	20	34	44	21	0	S	Mean
Location					**Kachemak Bay (AK)**					
Mean family yield (g replicate^{-1})	1,134.5^{a}	808.3^{ab}	871.3^{ab}	665.4^{ab}	659.1^{b}	646.4^{b}	687.2^{ab}	256.1^{c}	781.8	716.0
	(305.3)	(177.0)	(207.2)	(170.8)	(316.6)	(298. 8)	(365. 6)	(206.7)	(235.38)	(256.0)
Mean individual weight (g individual^{-1})	47.8^{a}	38.1^{abc}	41.6^{ab}	39.3^{abc}	39.1^{abc}	32.6^{bc}	38.7^{abc}	25.9^{c}	39.6	37.9
	(10.4)	(4.8)	(9.4)	(7.9)	(8.6)	(3. 6)	(5.3)	(6.6)	(7.73)	(7.1)
Mean survival (%)	68.1^{a}	61.4^{a}	60.0^{a}	49.1^{ab}	48.9^{ab}	54.8^{ab}	50.3^{ab}	26. 7^{b}	56.1	52.4
	(12.4)	(15.7)	(5.7)	(12.8)	(21. 8)	(23.7)	(27.0)	(17.1)	(7.5)	(17.0)
Location					**Thorndyke Bay (WA)**					
Mean family yield (g replicate^{-1})	4,670.0^{a}	4,317.5^{a}	5,305.0^{a}	6,180.0^{a}	7,525.0^{a}	4,301.3^{a}	4,766.3^{a}	5,514.2^{a}	5,295.0	5,322.4
	(2,610.5)	(1191.4)	(1,868.5)	(1,191.4)	(807.4)	(1,597.2)	(908.1)	(529.7)	(1,453.5)	(1,338.0)
Mean individual weight (g individual^{-1})	212.0^{a}	172.8^{abc}	198.1^{ab}	203.4^{a}	199.2^{a}	135.0^{a}	188.6^{ab}	150.4^{bc}	187.0	182.4
	(28.3)	(26.8)	(18.0)	(17.2)	(14.4)	(17.7)	(9.2)	(17.0)	(18.8)	(18. 6)
Mean survival (%)	43.5^{a}	53.0^{a}	52.7^{a}	61.0^{a}	75.5^{a}	63.5^{a}	51.0^{a}	73.7^{a}	57.2	59.2
	(23.3)	(26.3)	(14.7)	(11.9)	(5.0)	(20.0)	(12.3)	(6.3)	(16.2)	(15.0)

Standard deviation of the mean (SD). S Mean values for select MBP families not including control (0). Different superscript letters represent significant differences between families for each growth and biometric parameter (Tukey's Test, n = 12, 78 d.f., significance: $P < 0.05$). Differences were only tested for within sites and site interaction was not tested for families.

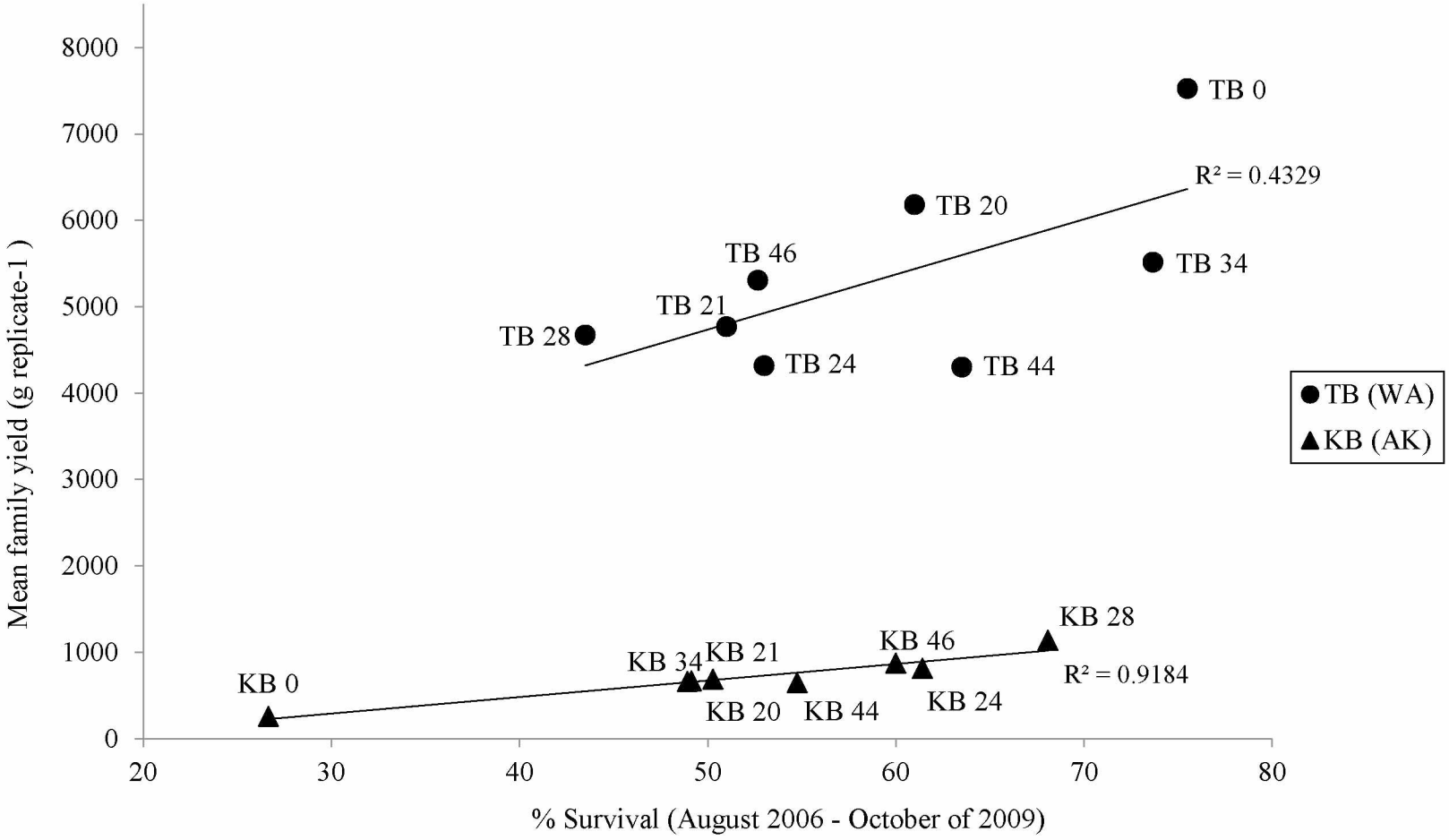

Figure 2.3. Percent family survival (October of 2006 – October of 2009) versus family yield (g family^{-1}) of families sampled from KB, AK [R^2 = 0.9184, P = 0.0053], and TB, WA [R^2 = 0.4329, P = 0.5119].

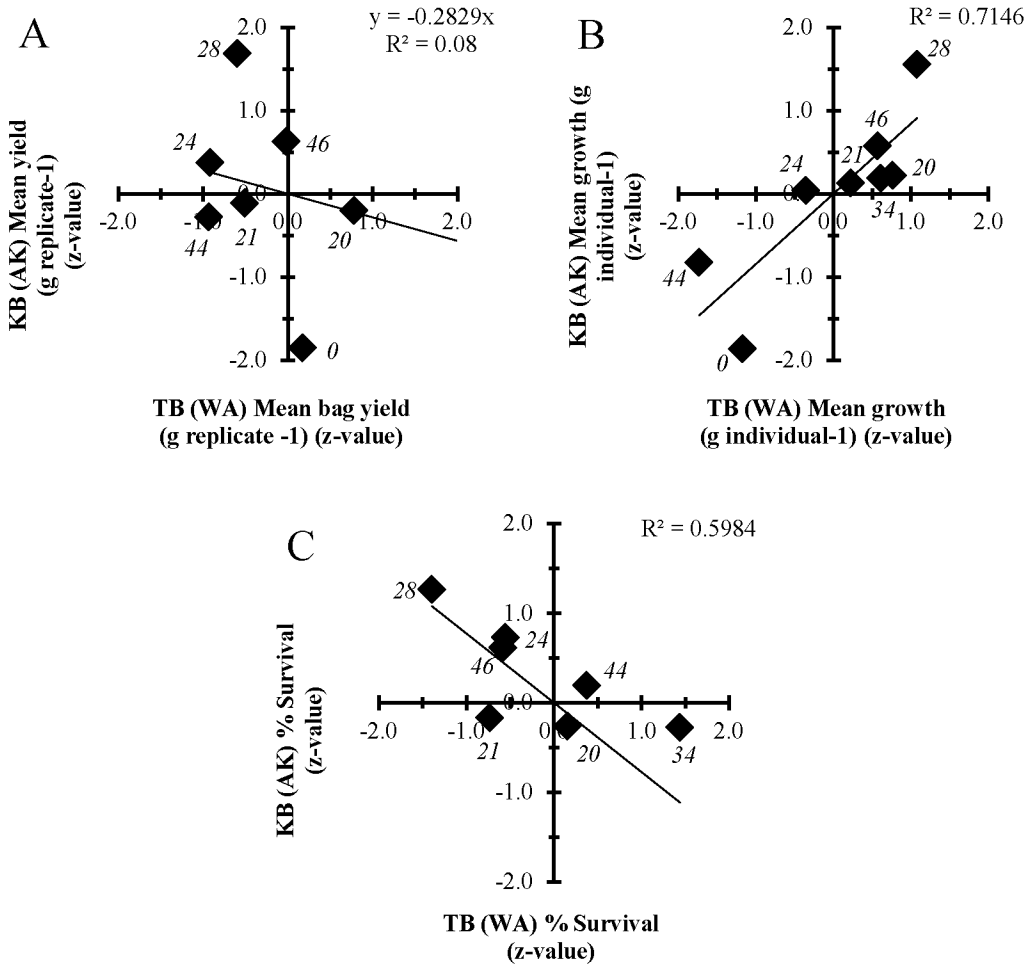

Figure 2.4. Standardized z-scores for biometric measurements of KB and TB oysters in October of 2009. (A) Mean family yield (g replicate^{-1}). (B) Mean individual growth (g individual $^{-1}$). (C) Mean family survival (%).

Table 2.3. Summary of comparisons of mean family yield, mean individual harvest weight, and mean survival of MBP families. Comparisons between sites and between top seven MBP selected families (S: *n* = 7 families) versus a control family (C: pooled group from *n* = 1 family).

Effects	Selection	Selection	Site
Site	**KB**	**TB**	-
	MBP Select vs. Control		**KB vs. TB**
Mean family yield (g replicate^{-1})	S > C (0.0001)	S = C (0.8734)	KB < TB (0.0000)
Mean individual harvest weight (g individual^{-1})	S > C (0.0001)	S = C (0.0854)	KB < TB (0.0000)
Mean survival (%) Aug 2006 - Oct 2009	S > C (0.0001)	S = C (0.2047)	KB < TB (0.0000)

Kachemak Bay (KB). Thorndyke Bay (TB). S: MBP families from selected broodstock. C: MBP controls from unselected broodstock. Statistical differences ($P < 0.05$). KB = TB no significant difference detected between oysters grown at KB and TB. KB > TB (P-value) parameter is significantly greater in oysters grown at KB than at TB.

Table 2.4. Biometric measurements and shell shape indices for MBP oysters sampled from KB, AK, and TB, WA, in October of 2009.

Family #		28	24	46	20	34	44	21	0	Mean
					Kachemak Bay (AK)					
Whole weight	(g)	50.4^{a}	41.8^{ab}	52.9^{a}	41.7^{a}	50.5^{ab}	38.9^{ab}	52.4^{a}	32.1^{b}	45.1
		(4.0)	(4.0)	(4.7)	(5.6)	(4.4)	(2.4)	(4.6)	(2.3)	(4.0)
Shell weight		37.0^{a}	29.6^{ab}	35.8^{ab}	27.5^{ab}	33.8^{ab}	27.3^{ab}	36.8^{a}	24.3^{b}	31.5
		(3.4)	(2.4)	(2.9)	(2.7)	(2.9)	(1.8)	(3.4)	(1.5)	(2.6)
Length	(mm)	89.3^{ab}	87.5^{ab}	89.9^{ab}	81.8^{ab}	93.0^{ab}	75.7^{b}	94.8^{a}	93.0^{a}	88.1
		(3.5)	(4.4)	(5.2)	(3.2)	(5.3)	(1. 5)	(4.0)	(5.3)	(4.0)
Width		63.7^{a}	72.3^{a}	67.9^{a}	68.8^{a}	66.7^{a}	67.1^{a}	66.0^{a}	66. 7^{a}	67.4
		(2.5)	(6.8)	(3.3)	(6.4)	(3.0)	(3.2)	(3.2)	(3.0)	(3.9)
Cup depth		27.7^{a}	24.6^{a}	25.6^{a}	25.0^{a}	24.6^{a}	32.3^{a}	24.0^{a}	24.6^{a}	26.1
		(1.4)	(1.2)	(1.2)	(1.8)	(1.1)	(4.6)	(0.9)	(1.1)	(1.7)
CI^{E} – Shape/size index		0.36^{a}	0.34^{a}	0.34^{a}	0.31^{a}	0.31^{a}	0.44^{a}	0.30^{a}	0.31^{a}	0.34
		(0.02)	(0.02)	(0.03)	(0.02)	(0.02)	(0.05)	(0.01)	(0.02)	(0.02)
CI^{B} W/L - Width/Length		1.36^{ab}	0.86^{a}	0.79^{ab}	0.73^{ab}	0.82^{b}	0.88^{a}	0.70^{b}	0.73^{ab}	0.78
		(0.03)	(0.09)	(0.06)	(0.03)	(0.06)	(0.03)	(0.04)	(0.03)	(0.05)
CI^{B} D/L – Depth/Length		0.27^{a}	0.30^{a}	0.31^{a}	0.27^{a}	0.28^{a}	0.42^{a}	0.26^{a}	0.27^{a}	0.30
		(0.02)	(0.02)	(0.04)	(0.02)	(0.01)	(0.05)	(0.01)	(0.02)	(0.02)
					Thorndyke Bay (WA)					
Whole weight	(g)	215.7^{a}	189.1^{a}	208.0^{a}	198.9^{a}	189.4^{a}	160.0^{a}	213.9^{a}	180.5^{a}	194.4
		(9.9)	(16.0)	(14.4)	(12.9)	(12.3)	(12.0)	(12. 8)	(14.9)	(13.1)
Shell weight		149.0^{a}	129. 8^{ab}	138.0^{ab}	138.6^{ab}	121.3^{ab}	104.7^{b}	145.8^{ab}	132.4^{ab}	132.4
		(7.5)	(11.3)	(10.8)	(8.6)	(7.6)	(8.8)	(7.8)	(11.2)	(9.2)
Length	(mm)	133.4^{a}	130.3^{a}	127.0^{a}	137.4^{a}	133.4^{a}	122.0^{a}	134.4^{a}	132.7^{a}	131.3
		(2.4)	(6.3)	(4.3)	(3.9)	(4.5)	(6.2)	(3.3)	(9.0)	(5.0)
Width		79.1^{a}	73.7^{a}	81.2^{a}	81.4^{a}	74.7^{a}	77.8^{a}	83.5^{a}	74.8^{a}	78.3
		(2.2)	(4.7)	(2.5)	(2.5)	(2.5)	(4.2)	(1.8)	(2.2)	(2.8)
Cup depth		44.0^{a}	39.3^{a}	41.7^{a}	34.1^{a}	42.3^{a}	39.4^{a}	42.1^{a}	38.5^{a}	40.2
		(1.6)	(2.0)	(2.7)	(3.4)	(4.1)	(4.2)	(1.8)	(1.7)	(2.7)
CI^{E} – Shape/size index		0.33^{b}	0.36^{ab}	0.34^{b}	0.32^{b}	0.33^{b}	0.35^{ab}	0.42^{a}	0.35^{ab}	0.35
		(0.01)	(0.02)	(0.01)	(0.01)	(0.01)	(0.01)	(0.04)	(0.02)	(0.02)
CI^{B} W/L - Width/Length		0.64^{a}	0.77^{a}	0.68^{a}	0.71^{a}	0.69^{a}	0.74^{a}	0.73^{a}	0.76^{a}	0.71
		(0.02)	(0.09)	(0.03)	(0.05)	(0.04)	(0.02)	(0.04)	(0.02)	(0.04)
CI^{B} D/L – Depth/Length		0.27^{b}	0.32^{ab}	0.29^{ab}	0.27^{b}	0.28^{b}	0.30^{ab}	0.36^{a}	0.31^{ab}	0.30
		(0.01)	(0.04)	(0.01)	(0.02)	(0.01)	(0.01)	(0.03)	(0.02)	(0.02)

Standard deviation of the mean (SD). Different superscript letters represent significant differences between families for each growth and biometric parameter (Tukey's Test, n = 12, 78 d.f., significance: $P < 0.05$). Differences were only tested for within sites and site interaction was not tested for families.

Table 2.5. Summary of comparisons of biometric data of MBP oysters, between sites, and between top seven MBP selected families (S: *n* = 7 families) versus a control family (C: drawn from *n* = 1 family) at the end of the growout period.

Effects	Selection	Selection	Site
Site	**KB**	**TB**	
Comparison	**MBP Select vs. MBP Control**		**KB vs. TB**
Whole weight (g)	S > C (0.0017)	S = C (0.2877)	KB < TB (0.0000)
Shell weight (g)	S > C (0.0075)	S = C (0.9850)	KB < TB (0.0000)
CI^E Shape/size index	C = S (0.4970)	C = S (0.7422)	KB < TB (0.0087)
CI^B W/L Width/Length	C = S (0.8170)	C = S (0.4436)	KB > TB (0.0000)
CI^B D/L Depth/Length	C = S (0.4510)	C = S (0.5504)	KB = TB (0.4601)
Length (mm)	S = C (0.2423)	S = C (0.8070)	KB < TB (0.0000)
Width (mm)	S = C (0.6887)	S = C (0.2387)	KB < TB (0.0000)
Cup depth (mm)	S = C (0.4650)	S = C (0.4340)	KB < TB (0.0000)

Kachemak Bay (KB). Thorndyke Bay (TB). S: MBP families from selected broodstock. C: MBP controls from unselected broodstock. Statistical differences ($P < 0.05$). KB = TB no significant difference detected between oysters grown at KB and TB. KB > TB (P-value) parameter is significantly greater in oysters grown at KB than at TB.

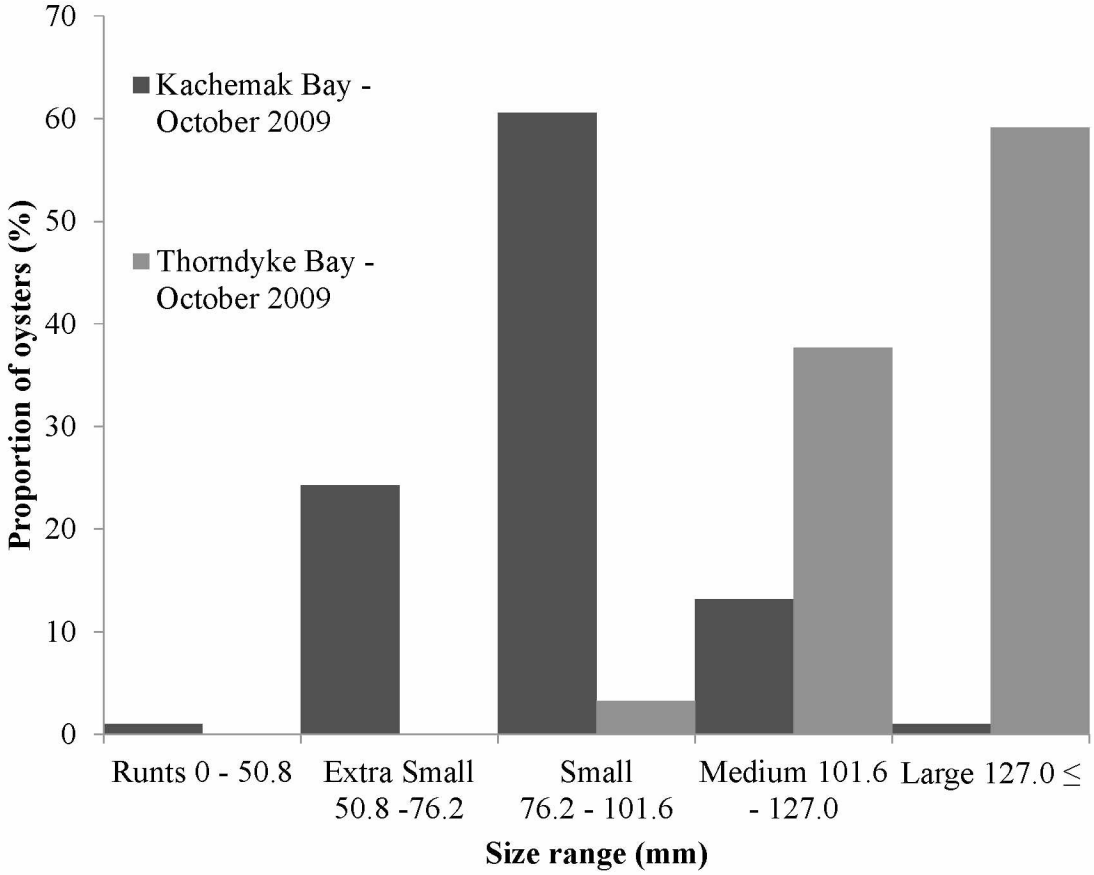

Figure 2.5. Distribution of MBP oysters by site within size categories as defined by RaLonde and Painter (1993) for AK oysters, at the end of the growout period.

Chapter 3: A seasonal comparison of post-harvest quality traits of selected families of Pacific oysters culture in Kachemak Bay, Alaska, and Puget Sound, Washington[2]

3.1. Abstract

Oysters grown in Alaska (AK) and destined for the half shell markets are renowned as a high quality product, but since 2005 the market for farmed oysters has seen a decline. The primary objective of this project was to characterize some biochemical features, oyster condition, and degree of reproductive development of families of oysters from the USDA funded Molluscan Broodstock Program (MBP) which relate to product quality. Oysters from the seven highest-yielding MBP and control families were grown at Kachemak Bay (KB), AK, in 2006 using suspended aquaculture. These were compared with siblings of the same families planted concurrently at an intertidal site in Thorndyke Bay (TB), Puget Sound, Washington. Oysters were sampled in October of 2009 and in June of 2010 at both sites. Biometric measures, proximate and fatty acid compositions, and reproductive development were compared. The differing biogeography and climate between the two sites led to differences in growth, reproductive development, and composition that together altered perceivable quality. Oysters grown in the cooler waters of KB, compared with TB, were slower growing with limited reproductive development, preserved glycogen as an energetic reserve for gametogenesis at the expense of protein, and had a higher ω-3 and ω-6 fatty acid content (particularly docosahexaenoic acid: 22:6ω3). Different latitudes were responsible for differences in physiology and composition,

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[2] Thomas, S.R., A.C.M. Oliveira, R. RaLonde, C.J. Langdon and G. Eckert. A seasonal comparison of post-harvest quality traits of selected families of Pacific oysters culture in Kachemak Bay, Alaska, and Puget Sound, Washington. This chapter has been prepared for submission in the Journal of Shellfish Research.

resulting in characteristically unique oysters from either site. Information from this study will be valuable for marketing Alaskan oysters and further development of breeding programs.

3.2. Introduction

Quality is the most important characteristic for the oyster consumer and determines their willingness to purchase (House et al. 2003, Harrington 2005). Visual perception of the opened oyster (Harrington 2005), quality and quantity of meat (Whyte & Englar 1982, Hand & Nell 1999), and taste (Conte et al. 1997, Harrington 2005) are essential product attributes to oyster buyers and consumers. The domestic and international oyster market differentiates individual oyster product by location. Growers, buyers, vendors, and consumers of Pacific oysters *Crassostrea gigas* (Thunberg, 1793) attest to the unique characteristics that oysters of this species may exhibit when grown in different localities. Indeed, culture location is often used as a product trademark (Brake et al. 2003).

Quality assessment of oysters requires determination of various physical, chemical, and sensorial characteristics. Condition indices are well-established measures of physical and intrinsic qualities of shellfish, serving also as economic or eco-physiological guides to quality (Lawrence & Scott 1982, Lucas & Beninger 1985, Crosby & Gale 1990). Condition indices reflect oyster biochemistry, reproductive biology, and growth, and can be correlated with growing conditions (Imai & Sakai 1961, Walne 1970, Gabbott & Stephenson 1974, Hand & Nell, 1999, Linehan et al. 1999) and other external environmental factors such as season (Linehan et al. 1999), temperature (Mann 1979), food availability (Whyte et al. 1990), water chemistry (Hochachka & Somero 1973), and genetics (Imai & Sakai, 1961, Evans & Langdon 2006, Hedgecock et al. 2006). Environmental changes such as temperature or feed availability influence the chemistry, biology, and physiology of the oysters. These changes can influence diverse functions such as feeding

behavior (Galtsoff 1964, Whyte et al. 1990), resource allocation (Soudant et al. 1999), and metabolic processes (Hochachka & Somero 1973, Bougrier et al. 1998). For instance, the reproductive strategy and gametogenesis of oysters, a dominant feature influencing quality of oysters, is inhibited as environmental temperatures decrease (Mann 1979, Berthelin et al. 1990, De La Parra et al. 2005). Gametogenesis involves the storage and allocation of nutrient reserves such as protein, glycogen, lipids, and minerals (Perdue et al. 1981, Whyte & Englar 1982, Soudant et al. 1999). Glycogen serves as an energy resource for gametogenesis, and lipid as major constituents of gametes (Mann 1979). As the oyster nears spawning, these energy resources deplete, resulting in a low condition and a poor-quality product (Conte et al. 1997, Mattes 2005). Spawning causes undesirable changes in product texture and flavor, likely perceived by consumers, that are detrimental to product quality and diminishes the market value. In turn this determines the optimal times for harvest and effective marketing. Detailed temporal quality information will assist in the development of oyster meat quality studies (Oliveira et al. 2006), marketing strategies (Harrington 2005) and best culture practices for the industry (RaLonde 1992, RaLonde & Painter 1993).

Dietary fatty acids are crucial to the biological functions of oysters. The fatty acid composition of their tissues can serve as indicators of diets utilized by oysters (Prato et al. 2010), as a tool for comparing the effect of location and seasonality on oyster composition (Linehan et al. 1999), as an indicator of the stage of their reproductive development (Linehan et al. 1999, Dridi et al. 2007), and as a nutritional guide to consumers considering healthy sources of essential fatty acids (Sargent 1997, Linehan et al. 1999, Oliveira et al. 2006). Furthermore, oyster fatty acid profiles, although subject to fluctuations, could be a useful tool in characterizing or 'trademarking' oysters by region.

In Alaska (AK), where oyster farms are generally located in remote areas with pristine water quality, farmers strive to produce a premium quality product that commands a top market value to offset high operational costs (RaLonde & Painter 1993, Harrington 2005). With the intent of boosting yields of Pacific oysters in AK, a long-term collaborative project was established between the USDA Molluscan Broodstock Program (MBP, Newport, Oregon [OR]), Alaska Sea Grant, the University of Alaska Fairbanks, and the Alutiq Pride Shellfish Hatchery (Seward, AK). The goal of the MBP was to gather oyster growth data from experimental growout sites placed on commercial farms, using oysters from pedigreed, selected broodstock that has shown high yields in AK, OR, and WA. The goal of this project was to take the research to the next step by collecting detailed quality data on oysters selected for high yield and comparing the data with that for unselected oysters. The availability of quality information in addition to growth data will benefit growers, vendors, and prospective breeding programs in AK, OR, and WA. This project adds an additional goal: to assess the quality of high yield broodstock from the MBP to assure continuance of premium oysters for the industry.

The goal of our study was to compare the quality of seven families of oysters grown with suspended culture at Kachemak Bay (KB), AK, and intertidally at Thorndyke Bay (TB), in Hood Canal, WA, and to compare their quality with that of control oysters derived from non-selected industry and wild stocks. These properties included meat quality, biochemical composition, fatty acid composition, and reproductive condition.

3.3. Materials and Methods

The growout sites in this study are located in areas with profoundly different climatic regimes and farming practices. In AK, oysters are grown exclusively using suspended, long-line, lantern net culture techniques; in contrast, WA oysters at Thorndyke Bay are grown in bags on the bottom, in the intertidal zone. The higher latitude of the growout site at Kachemak Bay (59

°34' N, 122°16' W) has a significantly colder water temperature regime throughout the year when compared to the lower latitude of the growout site at Thorndyke Bay (47°81' N, 122°66' W). Alaskan summers are short with long sunlight hours at mid-summer with water temperatures at Kachemak Bay never exceeding 20°C. The winters are long and the days short, the atmospheric and oceanic conditions are cold, and water temperature is relatively low throughout much of the winter, often reaching close to 0°C mid-winter. In WA, summer starts earlier and lasts longer, with shorter summer days but warmer water temperatures occasionally exceeding 20°C (Figure 3.2). Both Kachemak Bay (AK) and Hood Canal (WA) waters have good primary productivity suitable for oyster growth, but with differing diatom and dinoflagellate abundance and bloom timing regimes (Larrance et al. 1977, Strickland 1983, Field & Walker 2003, KBRR NERRS System Wide Monitoring Program 2010, NANOOS website 2011, NERRS website 2011). Pacific oysters will not spawn below 20°C, and require prolonged periods of water temperatures in excess of 10.5°C in order to generate and mature gametes (Mann 1979). The cool waters of the North Pacific typically suppress gamete development during the summer reproductive period, but primary productivity and hence food is readily available at both Kachemak Bay and Thorndyke Bay.

3.3.1. Environmental conditions

The MBP growout site at Kachemak Bay is located in Peterson Bay, near Homer, AK. A sheltered inlet extending ~2 km from the Southern coast of Kachemak Bay (59°58' N, -151°28' W) it is ~35 km east of the entrance of Kachemak bay into the Lower Cook Inlet which opens onto the Gulf of Alaska and circulating coastal currents. The bay lies opposite the Homer Spit which extends ~7 km into Kachemak Bay, where average bottom depth is ~30 m. The protruding Homer spit influences water circulation in the bay, as it acts as a partial barrier to currents and tidal drainage. Kachemak Bay is an estuarine environment, which receives oceanic nutrient-rich water

from localized upwelling in central lower Cook-Inlet (Muench et al. 1978, Abookire & Piatt 2005). Periods of stratification and mixing are dependent on wind, glacial melting, and precipitation that are determined by seasonality. The National Estuarine Research Reserve System (NERRS website 2011) collaborative water quality project website (http://www.nanoos-shellfish.org/Station-Data/Alaska/Homer-Ferry-Terminal-Dock/15.aspx) describes Kachemak Bay as 'a predominately estuarine environment during summer months when glacial runoff is highest, during the winter months reverting to a more marine-like system with glacial runoff at a minimum'. Kachemak Bay has a unique oceanographic condition in the form of a 'double gyre' system, which has the effect of increasing water retention times in the outer bay (Larrance et al. 1977). The increased retention time of waters in Kachemak Bay is a dominant factor contributing to large spring and summer phytoplankton populations. The result is productive water conditions suitable for oyster aquaculture operations, of which there are a number in the local area.

The MBP growout site in WA was located at Thorndyke Bay in the Hood Canal (47 °81'N, -122 °66'W). Thorndyke Bay is a small obtusely lunar shaped bay with a mixed and sandy/muddy bottom less than 1 km wide open to the Hood Canal. Thorndyke Bay is located in the central region of the Hood Canal ~ 70 km south of the entrance to the Juan De Fuca Strait which ultimately opens into the northwest Pacific Ocean ~ 120 km to the West. Water exchange in the deep fjord like system of the Hood Canal (up to 200 m deep) is slow and retention times high due to a large sill near the mouth and relatively long distance from the ocean. Waters in Hood Canal are susceptible to low dissolved oxygen concentrations and hypoxic conditions, due to high phytoplankton productivity in shallow waters and high levels of stratification resulting from low seawater flushing and freshwater run off (Hood Canal Dissolved Oxygen Program – HCDOP website 2011). Hood Canal is high in primary productivity. Blooms occur periodically, and are dependent on a complex relationship between freshwater runoff, circulation, upwelling at

deep-water sills, wind-mixing, and solar irradiation (Strickland 1983). The waters support multiple species of successful wild, and introduced commercial shellfish populations.

Surface temperature was continuously monitored at the Kachemak Bay growout site from the beginning of the growout period until May 10 of 2010, with a recorder (HOBO-TidbiT v. 2 Temp Logger, Pocasset, Massachusetts, USA) located on the Homer ferry dock at the end of the Homer Spit operated by NERRS. Any missing data from the Hobo-TidbiT temperature logger recording was supplemented using temperature data recorded with a data logger (HOBO-Water Temp Pro v. 2 Logger, Pocasset, Massachusetts, USA) sited in Peterson Bay at the Kachemak Bay Shellfish Nursery near the growout site across the bay from the Homer Spit. Daily temperature data were used to make a continuous temperature profile for the Kachemak Bay site over the experimental period.

Continuous water temperature monitoring did not occur at the TB site. A temperature profile was generated using data from a monitoring station in nearby Seattle, Puget Sound, WA. Hourly temperature data were taken from the National Oceans and Atmospheric Administration (NOAA), Tides and Currents Website, Meteorological Observations Station 9447130 (http://tidesandcurrents.noaa.gov/data_menu.shtml?stn=9447130%20Seattle,%20Puget%20Sound,%20WA&type=Meteorological+Observations). Daily temperature data provides a continuous temperature profile for Seattle, which showed a similar pattern to available discontinuous data of the temperatures observed at the experimental site in Thorndyke Bay (not shown). The data from Seattle was used as surrogate for comparison of water temperatures over the experimental period between the nearby Thorndyke Bay site and Kachemak Bay (see section 2.4.2; Fig. 2.2).

3.3.2. Experimental oysters

Seven of the highest-yielding families from MBP Cohort 20 were identified from a growout study which took place at Kachemak Bay and Thorndyke Bay and were used to assess

the quality traits of these families planted at both locations. Cohort 20 was created at the MBP hatchery at the Hatfield Marine Science Center, Newport, OR, in April of 2006 by crossing the highest yielding families from Cohorts 11 and Cohort 16 (Fig. 2.1), resulting in 45 families from selected broodstock. Four separate pairs of control families were also created (see below). All crosses, hatchery, and nursery husbandry techniques followed methods outlined by Langdon et al. (2003). On October 23 of 2006, 35 oysters from each Cohort 20 family were stocked into each of eight 3 mm mesh scallop spat collector bags. In contrast, WA oysters are grown on the bottom in the intertidal zone. Siblings from the same MBP and control families were concurrently planted at Thorndyke Bay, employing standard bag-on-bottom culture techniques (Langdon et al. 2003) with a stocking density of 50 oysters per bag. Fouling removal occurred at both locations in the spring and fall of 2007 using high-pressure water treatment at approximately 50 psi (345 kpa).

Initially, four separate pairs of control families were created to serve as "unselected" non-Cohort 20 control families by cross-breeding oysters from "wild" populations, oysters from sources within the industry, and from "standard" MBP broodstock. Each pair of control families was created from crosses of two separate families. Each control family was stocked between two replicated growout chambers at both experimental sites.

Control families 65 and 66 were created from crosses of Dabob Bay wild oysters. Dabob Bay is located in the Hood Canal, WA (47°83' N, 122°81' W) very close to TB (47 °81' N, 122 °66' W). The tideland at Dabob Bay is dominated by a sand substrate with spots of mud. It should be noted that the genetic profile of Pacific oysters categorized as 'wild' in WA are highly likely to be contaminated from cultured Pacific oyster stock grown in nearby culture areas. Control families 67 and 68 were wild crosses sourced from Taylor Shellfish Grower's culture site at Willapa Bay. Willapa Bay is located on the south WA coast (46°55' N, 124°0' W), and is a large, relatively shallow, estuarine inlet sheltered by a wide land spit. Oysters cultured in Willapa Bay

are grown in areas of sandy substrate, or on silty, muddy bottom. Control families 69 and 70 were created from crosses of Coast Seafood Company oysters between female oysters from Humboldt Bay, CA and male oysters from Dabob Bay, WA. Humboldt Bay is located on the coast of Northern California (40°74' N, 124°22' W). It is bar created lagoon, composed of clayey intertidal silts and sandy substrate. "Standard" control families 71 and 72 were created by crossing families formerly identified as average performers from MBP broodstock. These were Cohort 16 family 35 and Cohort 7 family 51, from the HMSC, Newport, OR.

On October 23 of 2010, 35 oyster seed from each Cohort 20 family and the eight control families were stocked into each of eight 3 mm mesh scallop spat collector bags. Mesh bags were placed in individual chambers of suspended lantern nets at Kachemak Bay. Seed from the same Cohort 20 and control families were concurrently planted at Thorndyke Bay, employing standard bag-on-bottom culture techniques (Langdon et al. 2003), with a stocking density of 50 oysters per bag. Fouling removal occurred at both locations in the spring and fall of 2007 using high-pressure water treatment at approximately 50 psi (345 kpa). Heavy losses at both KB and TB during the growout period resulted in the need to combine the four initially planned control families to form one group. Only families 65, 66 and 70 remained with any survivors from the initial 8 families intended for analysis. Families 65, 66 and 70 were pooled to create one control group, designated Family 0.

Sampling times were designed to take place at the end of the productive summer growth period in October of 2009 and in June of 2010 at the recommencement of productivity in spring and early summer (Whyte & Englar 1982, Oliveira et al. 2006). Oysters were sampled from the suspended lantern nets at KB on October 6 and 7 of 2009 and June 16 of 2010 and from the intertidal bags at TB on October 21 of 2009 and June 14 of 2010. At KB on October 6 of 2009

oysters from all MBP Cohort 20 Families were weighed and counted, and the top seven highest-yielding families were identified.

The top seven families and controls were cleaned of fouling and then sampled. For analysis of meat condition, four independent replicate bags were sampled for each family, with three oysters from each bag. A total of 96 oysters were sampled for assessment of condition at each time period for each site resulting in a total of 384 oysters. For the analysis of biochemical composition, limited availability of sample material resulted in three replicated samples per family, with each replicate consisting of four oysters. A total of 96 oysters were sampled at each time period for each site for assessment of biochemical composition. Samples were transferred from the growout sites in labeled net bags, stored in wet-lock boxes, and transported overnight to the Fishery Industrial Technology Centre in Kodiak, AK, where they were refrigerated and shucked for frozen storage within 48 h.

3.3.3. Condition indices

In order to assess meat quality, oyster researchers utilize an index determined from standardized multiple biometric measurements of meat weight, whole weight, and shell weight. The index used in this project was developed by Hand & Nell (1999) by combining methods of Lawrence & Scott (1982) and of Crosby & Gale (1990); this index is known as the dry meat condition index (CI^{HN}). Twelve oysters from each selected family or the control family were split to create four replicates comprising three oysters to be used in the calculation of the CI^{HN}. Oysters were shucked, and the meats and juices separated from their shells and collected together. Meat and juices were drained and placed together into a pre-weighed 200 mm aluminum-weighing dish (VWR International, Brisbane, California, USA). Shells were also placed into a pre-weighed 200 mm aluminum-weighing dish (VWR). Individual shell weight and the weight of oyster meats and juices were recorded. The data was used to compute the CI^{HN} index as:

$$CI^{HN} = dry\ meat\ weight\ (g) \times \frac{1000}{cavity\ volume}$$

Equation 3.1

(Hand & Nell 1999).

$$cavity\ volume = whole\ weight\ (g) - \ shell\ weight\ (g)$$

Equation 3.2

(Lawrence & Scott 1982).

In order to determine dry meat weight, pre-weighed oyster meats and juices, previously separated from the shell and placed in aluminum dishes, were placed in a drying oven at 105°C for 48 h. After 48 h dried samples were removed and allowed to cool in glass desiccators (Hand & Nell 1999), then weighed using a top loading scale with manufacturer accuracy of 0.01g to determine final dry weight.

3.3.4. Biochemical composition

The biochemical analysis of oysters was conducted according to the scheme of Linehan et al. (1999) and Oliveira et al. (2006). Twelve oysters were randomly selected from each family; three replicated pools were created with four oysters each. Oysters were shucked and meat and shell cavity fluids were placed in screw-top jars with shell pieces and grit carefully excluded. Pooled contents of the jars were homogenized for 1 min using a blender (Model 31BL92, Waring, Torrington, Connecticut, USA), frozen in vacuum bags, vacuum-sealed, and stored at -80°C until analysis.

3.3.4.1. Proximate composition

Total solids were determined by AOAC method 952.08B (AOAC 1990) using 2 g of homogenized sample for each replicate, and presented as a percentage of the total wet weight of oysters solids and interpalial fluids. Percent ash was determined in accordance with AOAC

method 938.08 (AOAC 1990), using the 2 g of homogenized meat for each replicate sample which had been used to determine total solid content; thus, the dry samples were placed in a furnace at 550°C for 24 h and ash content determined gravimetrically. Protein content of the oysters was quantified using wet tissue, with a nitrogen analyzer (Model FP2000, LECO, St. Joseph, Michigan, USA) as previously reported by Oliveira et al. (2006). Lipid extraction was performed using an accelerated solvent extraction system (ASE 200; Dionex, Sunnydale, California, USA) as described in Oliveira et al. (2006). Total carbohydrate content was determined with wet tissue, using the combined methods of Strickland & Parsons (1968), and Clegg (1956), commonly referred as the "Anthrone Method" as described by Oliveira et al. (2006). A quantity of 2.5g of homogenized sample was used for each analysis. A volume of 10 mL distilled water was added to a 100 ml volumetric flask together with 13 mL of 52% perchloric acid. The flask was capped and the contents stirred for 30 min at room temperature on a standard laboratory stir plate (VWR International, Brisbane, California, USA). The mixture was then diluted to the 100 mL mark and filtered into a 250 mL volumetric flask. The filtrate was diluted to the 250 mL mark; a 10 mL portion was transferred to a 100 mL volumetric flask and further diluted to the 100 mL mark. A volume of 1 mL was then transferred to a 25 mL screw-top tube for the spectrophotometric analysis. The anthrone reagent was prepared with 0.2 g of anthrone (Aldrich, Milwaukee, WI, U.S.A.) in 8 Ml of HPLC grade ethyl alcohol (VWR) and 30 mL distilled water. After complete dissolution of the anthrone in the solvents, 100 mL concentrated sulfuric acid was added to the mixture. A quantity of 5 mL anthrone reagent was added to the 1 mL sample in the 25 mL screw-top tube, which was placed in a boiling water bath for 7 min. The tubes cooled quickly and absorbance at 624 nm was read immediately on a Hewlett Packard 8452A diode array spectrophotometer (Agilent Technologies, Wilmington, Delaware, USA). Distilled water was used a blank and a solution of 1 mL of 0.1 mg of oyster glycogen (Aldrich) in

distilled water was used as standard for the spectrophotometric analysis. Calculation of total carbohydrate, which in oysters is present mostly as glycogen (Walne 1970), used the weight of the sample (W), the absorbance of oyster glycogen standard (A_1), and the absorbance reading of digested and diluted sample (A_1) (Equation 3.3). The results describing all of the biochemical components of the oysters were presented as a percentage of the dry (solid) weight.

$$\% \, glycogen = \frac{(25 \times A_2)}{(A_1 \times W)}$$

Equation 3.3

3.3.4.2. Fatty acid composition

Extracted lipids for each family were combined and fatty acid methyl esters (FAME) determined and mean values averaged for triplicate sub-sample. The FAMEs were prepared according to the procedure of Maxwell and Marmer (1983), with methyl tricosanoate used as the internal standard. Fatty acid methyl esters were quantified as described by Oliveira et al. (2006) using a Gas Chromatograph (GC) model 6850 (Agilent Technologies) fitted with a DB-23 (60 m x 0.25 mm id., 0.25 μm film) capillary column (Agilent Technologies) coupled to a flame ionization detector (FID). The chromatographic data was collected and analyzed using the GC ChemStation program (v. A.08.03; Agilent Technologies). Hydrogen was used as carrier gas at linear flow of 1.0 mL/min with an average velocity of 29 cm/s. The initial nominal pressure of the inlet was 100.3 kPa, and both injector and detector were held at 275° C. The split ratio was set to 50:1 and the oven programming was carried out as follows: initial temperature was set to 140°C and increased to 180°C at 2°C/min, from 180 - 200°C at 2.5°C/min, 200 - 210°C at 0.5°C/min, and from 210 - 230°C at 10°C/min for a total run time of 60 min. The detector was operated at a constant makeup flow of 35 mL/min of nitrogen, with an air and hydrogen flow of 450 mL/min

and 40 mL/min, respectively. An autosampler performed the GC injections of standards and sample, and injection volume was set at 1 μL. The ChemStation Enhanced Integrator program was used to integrate the chromatogram peaks. All standards used in the identification of peaks were purchased from Supelco® (Supelco Park, Bellefonte, Philadelphia, USA). Selected standards used in this analysis were: Supelco 198-19, Bacterial Acid Methyl Esters Mix, Marine Oil #1, and Marine Oil #3. The quantities of saturated, monounsaturated, omega-3, omega-6, and polyunsaturated fatty acids were determined based on the entire fatty acid profile of the oysters. Results from the three most abundant fatty acids were statistically compared and are discussed, while the remaining fatty acids of lower abundance are presented without further analysis. Results were expressed as percent of total fatty acids in the oyster lipid extracts. Fatty acid composition data is only available for those oysters sampled from KB and TB in June of 2010.

3.3.5. Reproductive condition

Twelve oysters per family were sampled from KB and TB in both October of 2009 and June of 2010 for analysis of reproductive condition. Due to limited sample availability of oysters in October of 2009 at TB only nine animals were available from MBP family 46, and at KB in June of 2010 only six animals were available from MBP family 21. Oysters were shucked, the body separated from the shell and placed flat on a cutting board. Sample sections were obtained as described in the procedures developed by Ellis et al. (1998) for gonadal analysis of bivalves. Sections were immediately submerged and stored in labeled screw top vials containing Davidson's fixative (Poly Scientific, Bay Shore, New York, USA) until further analysis. Samples were drained of Davidson's fixative for transport to the Hatfield Marine Science Centre (HMSC), Oregon State University (OSU) in Newport, OR. Upon arrival at the facility samples were submerged in 70% ethanol for safe handling during image analysis and to dehydrate samples for histological analysis.

The oyster body sections were laid flat in glass culture dishes and dabbed with a tissue to removed residual fluid. Images were captured using a Nikon digital camera (Model D40), mounted on a light stand at a height of 32 cm. The lens was set at maximum zoom (55x), and camera flash was used. Sections were centered in the middle of the image for capture with a 1 cm x 1 cm grid background. Images were individually adjusted for contrast and brightness using a sliding scale tool in Microsoft Windows Photo Gallery (v. 15. 2010, Microsoft Corporation, Redmond, Washington, USA). Gonad areas of transverse sections of oyster bodies were determined using a 'polygon' drawing tool in Image-Pro Plus image analysis software (v. 4.5.1. 2003, Media Cybernetics Inc., Silver Springs, Maryland, USA). Area of gametes is presented as the relative area of differentiated gonadal material combined (Fig. 3.1) to the area of the whole visceral mass (Ellis et al. 1998).

Gonadal sections of twelve oysters with different observed areas of gonad development were sent to Dr. Ralph Elston (AquaTechnics, Washington, USA) so that we could correlate visual estimates of gonadal development with histological measures of gametogenic development. Stages of gametogenic development were histologically categorized as follows: undifferentiated gametes (stage 0), developing gametes (stage 1 and 2), and ripe gametes (stage 3), based on criteria proposed by Mann (1979), and by Steele & Mulcahy (1999). Different stages of gametogenic development, from undifferentiated (stage 1) to ripe gametes (stage 3) showed significant positive correlation ($R^2 = 0.81$, $P < 0.05$) with the visually measured gonad area, with gonad areas ranging from 29.3% to 56.7% of the visceral mass.

3.3.6. Data analysis

Using 44 mean monthly water temperatures from KB and Seattle, an unpaired Student's T-test was used to compare the two locations for differences (87 degrees of freedom). All recorded data were summarized at each site and sample time, and averages and standard

deviations were determined from replicate measurements of each. Percentage data (y) were transformed using an arcsine transformation to centralize the data about the mean to increase the normality of each distribution, and was calculated as follows:

$$Sin^{-1}(\sqrt{y})$$

All statistical tests were performed using Statistica (v. 9.0, Statsoft, Tulsa, Calif., USA). In order to conduct Analysis of Variance (ANOVA: 95%; confidence: $P < 0.05$), data were tested for normality using a Shapiro-Wilks test. One-way ANOVA was applied to data that were normally distributed (Shapiro-Wilks: 95%; confidence: $P < 0.05$).

In the cases when normality requirements were not met, a nonparametric Kruskal-Wallis test (K-W) was applied. An *n* of 96 oysters comprised a sample replicate within a site or season (one degree of freedom), and for an individual family (total of 8 families including the control) an *n* of three replicate groups of four individuals was used (15 degrees of freedom). The significant differences of individual quality parameters between selected MBP families and the control family of oysters, sampling sites, sampling times and between individual families were determined using a Tukey's Honest Significant Differences Test (ANOVA: Post-Hoc, 95% confidence, $P < 0.05$).

3.4. Results

The results of analysis for the sets of post-harvest quality traits of interest are presented in the following subsections. The individual sample sites where oysters were grown using suspended net culture techniques at KB and using intertidal bag culture at TB are described separately. Within the description of results at each site are the comparisons between the control family of oysters and the mean of the selected families of MBP oysters for each attribute. A seasonal comparison is also made between the first sampling in October of 2009 and the second

sampling in June of 2010 for each site. The final subsection for each set of attributes provides comparisons between sites and individual family differences within sites.

3.4.1. Environmental conditions

Continuous temperature data (°C) for KB site are found in Fig. 3.2 from October 25 of 2006 to October 10 of 2010, and for Seattle, Puget Sound, WA, from October 25 of 2006 to June 15 of 2010. Both KB and Seattle show a distinct seasonal cycle in water temperatures.

3.4.1.1. Kachemak Bay

At KB, the mean annual temperature between January of 2007 and May of 2010 was 6.2 ± 3.1°C, the maximum temperature was 12.2°C and was reached on July 3 of 2009, and the minimum temperature of 0.5°C was reached on March 15 of 2007.

3.4.1.2. Thorndyke Bay

At the Seattle observation station, the mean annual temperature between January of 2007 and May of 2010 was 10.4 ± 1.8°C, the maximum temperature at KB (13.9°C) was reached on July 4 of 2009, and the minimum temperature of 7.5°C reached on March 19 of 2008. Temperature monitoring data available from the University of Washington School of Oceanography website, from nearby Dabob Bay in the Hood Canal, reported surface temperatures as high as 20°C in mid to late June and onwards in the summers of 2007 to 2009 (NANOOS website 2011).

3.4.1.3. Site temperature comparisons

Mean annual temperatures in Puget Sound, WA were significantly warmer than at KB, AK, in each year from 2006 to 2009 (T-test: $P < 0.05$; Fig 2.2). The mean temperature for the entire period 2006-2010 was also significantly higher in WA than in AK ($P < 0.05$). It is

important to consider that oysters growing in the intertidal zone would experience the effects of ambient air temperatures during the twice-daily exposure of low tide, as well as solar heating effects during daylight low tides, but this study did not monitor these micro-scale environmental factors.

3.4.2. Condition indices

3.4.2.1. Kachemak Bay oysters

Mean meat weight of all families at KB was 11.8 ± 1.5 g and cavity volume was 13.6 ± 2.3 g in October of 2009 (Table 3.1), and 12.9 ± 5.4 g and 14.4 ± 5.9 g in June of 2010 (Table 3.2). Meat weight and cavity volume among selected MBP families of oysters were significantly higher than the control family at KB October of 2009, but were similar for both measurements in June of 2010 ($P < 0.05$; Tables 3.1 and 3.2). Meat weight and cavity volume was similar at KB between October of 2009 and June of 2010. At KB in October of 2009 the mean CI^{HN} was higher ($P < 0.05$) in the control family of oysters than the mean value determined for the selected MBP families, whereas there were no differences observed between the selected families and the controls in June of 2010. The CI^{HN} was higher ($P < 0.05$) among oysters sampled in October of 2009 (153.5 ± 13.3) than it was in June of 2010 (149.2 ± 10.0). These differences suggest that overwintering led to a reduced meat fill of the oyster cavity at KB.

3.4.2.2. Thorndyke Bay oysters

Mean meat weight of all families at TB was 62.2 ± 5.0 g and cavity volume was 62.1 ± 5.0 in October of 2009 (Table 3.1), and 53.5 ± 13.4 g and 55.3 ± 13.8 in June of 2010 (Table 3.2). Meat weight and cavity volume among selected MBP families of oysters were significantly higher than the control family at TB October of 2009 but were similar for both measurements in June of 2010 ($P < 0.05$; Tables 3.1 and 3.2). At TB meat weight was higher in October of 2009

than in June of 2010. However, cavity volume was similar at TB between sampling times ($P > 0.05$; Tables 3.1 to 3.3). At TB in October of 2009, the mean CI^{HN} was significantly higher ($P < 0.05$) in the control family of oysters than the mean observed value for the selected MBP families, but there were no such differences observed between the selected families compared to the control family in June of 2010. At TB, the CI^{HN} was significantly lower ($P < 0.05$) among oysters sampled in October of 2009 (129.2 ± 7.7) than it was in June of 2010 (181.5 ± 8.4). After winter, by June of 2010, oysters had good meat fill within the shell cavity.

3.4.2.3. Site differences and MBP family comparisons

Meat weight and cavity volume was significantly lower at KB than at TB in both October of 2009 and June of 2010 (Table 3.3). There were no significant differences in meat weight or cavity volume among MBP families at KB or TB in October of 2009 ($P > 0.05$; Table 3.1). All families at KB had similar meat weight in June of 2010, while family 46 had a greater cavity volume than the control family ($P < 0.05$; Table 3.2). At TB, cavity volumes were similar among families in 2009, while in June of 2010 family 28 had a larger mean cavity volume than the control family (Table 3.2).

The CI^{HN} was similar ($P > 0.05$) at KB and TB in October of 2009, but significantly lower ($P < 0.05$) at KB than TB in June of 2010. At KB and TB in 2009, comparison of the CI^{HN} between individual families within sites in October of 2009 showed no differences ($P > 0.05$), suggesting that meat fill of the cavity of the oysters did not vary between MBP families at either site (Table 3.1). The same was true of CI^{HN} at KB in June of 2010, while family 28 was significantly higher than the control at TB ($P < 0.05$; Table 3.2).

3.4.3. Biochemical composition

3.4.3.1. Proximate composition

3.4.3.1.1. Kachemak Bay oysters

The mean solids content of all oysters sampled in October of 2009 were significantly higher ($P < 0.05$; 16.59 ± 0.5 % wet wt.) than that of oysters sampled in June of 2010 (13.9 ± 1.3 % wet wt., Table 3.4). The mean solids content of the control family of oysters did not differ ($P > 0.05$, Table 3.5) from values determined for selected families of MBP oysters grown at KB in either October of 2009 or June of 2010. The results indicated that solid content did not vary between selected MBP families and the control family of oysters, but that overwintering led to a reduction in total solids.

Of the constituents of the solid fraction of the oysters from KB, protein was the most abundant, followed in lessening order by glycogen, ash, and lipids (Table 3.4). In October of 2009, the proximate composition of the control family of oysters was similar to the average composition of the selected families of MBP oysters (Table 3.4). In June of 2010, the only significant difference observed in the proximate composition between control family and the average values for the top seven MBP families was protein content, which was higher in the control family (Table 3.5). The mean protein and glycogen contents among oysters grown at KB were 50.1 ± 1.7 % and 27.0 ± 1.4 % dry wt. (Table 3.4). In October of 2009 and 42.6 ± 1.9 % and 34.6 ± 2.6 % dry wt. in June of 2010, respectively. The protein content of oysters at KB was higher in October of 2009 than in June of 2010 ($P < 0.05$) while glycogen content was lower in October than in June (Table 3.5). With regards to minor components, the ash content was similar ($P > 0.05$) at KB in October of 2009 (13.5 ± 0.9 % dry wt.) compared to June of 2010 (13.7 ± 2.0 % dry wt.), and the same was true of the lipid content in October of 2009 (9.4 ± 2.5 % dry wt.) and June of 2010 (9.1 ± 0.7 % dry wt.). Overall, few differences were observed in the proximate

composition of the control family and selected MPB families for each sampling period. However, compositional changes in protein and glycogen were recorded with respect to season.

3.4.3.1.2. Thorndyke Bay oysters

The mean solids content of control family oysters did not differ ($P > 0.05$, Table 3.5) from values determined for selected families of MBP oysters grown at TB in either October of 2009 or June of 2010. However, the average solids content of all oysters sampled in October of 2009 were significantly lower ($P < 0.05$; 15.4 ± 0.6 % wet wt., Table 3.4) than of oysters sampled in June of 2010 (19.2 ± 0.9 % wet wt.). These results show the influence of season in the content of solids of oysters grown in TB, which far exceed variability of this measurement between selected MBP families and the control family.

Among constituents of the solid component of the oysters from TB, protein was the most abundant component followed by glycogen and ash, with lipids being the least abundant. The mean protein and glycogen contents among oysters grown at TB were 51.5 ± 2.7 % and 24.5 ± 2.0 % dry wt. in October of 2009 and 58.8 ± 1.4 % and 22.7 ± 1.4 % dry wt. in June of 2010, respectively. Significant differences ($P < 0.05$) were observed for protein contents of oysters with respect to season but glycogen content did not vary ($P > 0.05$). With regards to minor components, the mean ash content was higher ($P > 0.05$) in October of 2009 oysters (15.4 ± 1.2 % dry wt.) than in June of 2010 oysters (9.4 ± 0.2 % dry wt.). Lipid content was similar ($P > 0.05$) at TB in October of 2009 (8.6 ± 0.9 % dry wt.) compared to June of 2010 (9.1 ± 0.6 % dry wt.).

Overall, few differences were observed in the proximate composition of the control family and selected MPB families for each sampling period. However, compositional changes were recorded with respect to season.

3.4.3.1.3. Site differences and MBP family comparisons

The solids content of oysters grown at KB was significantly lower ($P < 0.05$) than that of oysters grown at TB in October of 2009, while the opposite was observed for June of 2010. In October of 2009, the protein content of oysters grown at KB was similar to those grown at TB ($P > 0.05$); however, in June of 2010 protein contents differed ($P < 0.05$) and KB oysters had lower values. In both October of 2009 and June of 2010, glycogen content was significantly higher ($P < 0.05$) among KB oysters when compared to those grown at TB. In October of 2009, the ash content of oysters was lower ($P < 0.05$) at KB than at TB, whereas the opposite was true in June of 2010. The lipid content of oysters grown at KB was similar ($P > 0.05$) to that of those grown at TB in both October of 2009, and June of 2010 (Table 3.4 and 3.5).

At KB in October of 2009, family 28 oysters had significantly greater ($P < 0.05$) solids content than families 21, 46, 34, and the control. Furthermore, in June of 2010, the solids content of family 28 was greater than that of family 21 (Table 3.4). At TB, in October of 2009 family 34 had significantly lower solids content than both the control and family 20, whereas there were no differences among families in June of 2010. At KB in October of 2009, oyster families were generally similar ($P > 0.05$) in proximate composition. Only family 34 was determined to have higher ash content ($P < 0.05$) than that of family 28 at KB. The same was generally true of the composition of oysters at KB in June of 2010, with the exception of the protein content which was lower ($P < 0.05$) in family 28 than in the control family. At TB in October of 2009, family 34 oysters had lower solids content than the control or family 20 ($P < 0.05$) but in June of 2010 there were no differences among families ($P > 0.05$). There were no differences observed between families in the composition of the solids ($P > 0.05$) at TB in October of 2009. However, in June of 2010, families 44 and 20 were higher in ash than of families 24 and 34. In addition, family 44 was higher ($P < 0.05$) in protein than both family 24 and 34 (Table 3.4). This meant

that the glycogen content of family 44 was lower than those of family 24 and 34. Family 44 also had lower lipid content than that of the control family. The observed differences in the composition of individual families of oysters were also influenced by different growing locations and culture type.

3.4.3.2. Fatty acid composition

3.4.3.2.1. Kachemak Bay oysters

Polyunsaturated fatty acids (PUFA) were the most abundant fatty acid class in KB oysters, followed by saturated fatty acids (SFA), and monounsaturated fatty acids (MUFA). The PUFA content was on average 50.1 ± 0.6 % of the total fatty acids, while SFA content averaged 23.3 ± 0.5 % and MUFA content was 18.6 ± 0.3 % (Table 3.6). The SFA and PUFA content of oyster families at KB were similar between selected oysters and control oysters ($P > 0.05$, Table 3.7) at KB in June of 2010.

Within the PUFA class, the content of omega-3 FA was 46.3 ± 0.5 % of total FA. In the omega-3 FA class, the most abundant FA were the long chain highly unsaturated fatty acids, docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA). The content of DHA was 16.1 ± 0.5 % total FA and EPA was 24.1 ± 0.7 % total FA. Of the omega-6 FA the most abundant was 18:2 ω 6 *cis* or linoleic acid (2.3 ± 0.1 % total FA). The most abundant SFA was 16:0 or palmitic acid (16.2 ± 0.4 % total FA), which when combined with DHA and EPA, contributed to 56.4 % of total FA. The other nine FA presented in Table 3.8 account for 29.8 % total FA, each individual FA ranging from 1% to 5% of the total FA in the lipid extracts. Generally, the control family of oysters and the selected MBP oysters were similar in their fatty acid classes and individual FA at KB ($P > 0.05$), with the exception of palmitic acid in which the control family was higher than in the selected group of families ($P < 0.05$).

3.4.3.2.2. Thorndyke Bay oysters

In oysters from KB, the PUFA were the most abundant FA class followed by SFA and then MUFA, the same pattern as observed at TB. The distribution of fatty acid classes was similar ($P > 0.05$) between the control family of oysters and the selected families of MBP oysters grown at KB in June of 2010 ($P > 0.05$), with the exception of the MUFA content that was lower in the control family ($P < 0.05$). At TB, the PUFA content averaged 45.1 ± 0.8 % of the total FAs, while SFA content averaged 26.3 ± 0.8 % total FA, and MUFA content was 22.2 ± 0.3 % total FA.

Of the PUFA, the content of omega-3 FA was on average 41.9 ± 0.8 % of total FA. In the omega-3 fatty acids class, the most important FA were the long chain highly unsaturated fatty acids, docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA). The content of DHA was 13.2 ± 0.6 % total FA, and EPA was 24.5 ± 0.4 % total FA. Of the omega-6 FA, the most abundant was 18:2 ω 6 *cis* or linoleic acid (1.2 ± 0.1 % total FA). The most abundant SFA was 16:0 or palmitic acid (16.4 ± 0.6 % total FA), which when combined with DHA and EPA contributed to 54.1 % of total FA. The nine remaining fatty acids (> 1% of total FA), shown in Table 3.8, account for 28.1 % of total FA and each individual FA ranged from 1% to 5% of total FA in the samples. The control family of oysters and the collective mean of the selected MBP families were similar in terms of fatty acid classes and individual FA at TB ($P > 0.05$). However, individual families did vary from the control family.

3.4.3.2.3. Site differences and MBP family comparisons

In June of 2010, the PUFA content of KB oysters was higher than those of TB oysters, while the opposite was true of the SFA and MUFA content. The omega-3 and omega-6 FA were higher in oysters grown at KB than those of oysters grown at TB ($P < 0.05$). Palmitic and EPA

contents were similar at both locations ($P > 0.05$); however, the DHA content was significantly greater in oysters grown at KB than those of oysters grown at TB ($P < 0.05$).

Levels of DHA, EPA, and palmitic acid were similar among all families ($P > 0.05$). Slight, yet non-significant ($P > 0.05$), differences were observed between selected oysters and the control family grown at KB and at TB. Selection and site both influenced FA composition of oysters studied.

3.4.4. Reproductive condition

Using the results of Dr. Ralph Elston's histological analysis performed on the sub-set of twelve samples sent to Dr. Ralph Elston, we observed that different numeric stages of gametogenic development, from undifferentiated (stage 1) to ripe gametes (stage 3) showed significant positive correlation ($R^2 = 0.8139$, $P = 0.0455$) with the visually measured gonad area, with gonad areas ranging from 29.3% to 56.7% of the visceral mass. Based on this significant and strong correlation we considered that the visual estimates of gonadal development were an acceptable representation of sexual maturity in the oysters.

3.4.4.1. Kachemak Bay oysters

At KB, the percent of total gonad area occupying the cross-sectional visceral mass of the control family was similar ($P > 0.05$) to the values determined for selected families of MBP oysters when sampled in October of 2009 or June of 2010 (Table 3.9). The total gonad area of oysters grown at KB (Figure 3.3) was significantly lower ($P < 0.05$; Table 3.9) in October of 2009 (40.2 ± 13.8 %) than in June of 2010 (50.3 ± 7.8 %).

3.4.4.2. Thorndyke Bay oysters

At TB, the percent of total gonad area occupying the cross-sectional visceral mass of the control family was similar ($P > 0.05$) to the values determined for selected families of MBP

oysters in October of 2009 and June of 2010 (Table 3.9). The total gonad area of oysters grown at KB was significantly higher ($P < 0.05$; Table 3.9) in October of 2009 (44.4 ± 7.2 %) than in June of 2010 (34.6 ± 10.3 %). As at KB, the results for TB indicate that seasonality has an influence on degree of reproductive development. These patterns were the same as those observed at KB. The results dictate that selection has not influenced the degree of reproductive development in MBP oysters at TB.

3.4.4.3. Site differences and MBP family comparisons

The total gonad area occupying the visceral mass was significantly lower ($P < 0.05$) among oysters grown at the higher latitudes of KB when compared to the lower latitude site of TB in both October of 2009 and June of 2010 (Table 3.9), suggesting less reproductive development was occurring in oysters grown at KB than at TB. At KB in October of 2009, the total gonad area was significantly greater in oysters of family 44 and 28 when compared to family 20. The gonadal area of family 44 was also higher than family 21 and the control family ($P < 0.05$). In June of 2010, there were no differences in gonad area among families ($P > 0.05$). At TB, there were no differences detected in the gonad area of oysters in October of 2009 or June of 2010. It would appear that selection and site influenced the reproductive development to different degrees among families grown at KB.

3.5. Discussion

MBP oysters from selected broodstock and their intrinsic quality attributes show a number of significant differences between the two sampling sites (KB and TB), as well as changes during the over winter period from October of 2009 to June of 2010. Ultimately, the growth, biological, metabolic, and biochemical rhythms of the families grown at the two different

sites are varied as a result of the changes in the physiology of Pacific oysters in response to a widely differing environmental conditions.

3.5.1. Condition indices

The dry meat index indicates the level of condition. An oyster with a dry meat condition index of 100 is considered to be in 'excellent' condition, and values between 80 and 100 represent oysters in 'good' condition (Westley 1954, Oliveira et al. 2006). At KB and TB, over 78% of oysters were in excellent condition in October of 2009 and June of 2010.

3.5.1.1. Kachemak Bay oysters

Mean values of CI^{HN} of oysters grown at KB sampled in October of 2009 and June of 2010 (Tables 3.1 and 3.2) were greater than values reported by Oliveira et al. (2006) for oysters sampled from KB in October of 2003 (123.1) and June of 2004 (119.7). There has been a decline in mean water temperatures over the ten-year period 2000 to 2010 (Personal communication: Ray RaLonde 2009; unreferenced). The reduction in CI^{HN} observed over winter between October of 2009 and June of 2010 at KB (Tables 3.1 and 3.2) follows a similar pattern to that observed by Oliveira et al. (2006) at KB in June of 2004, and is possibly a function of lower condition resulting from the relatively cooler temperatures in recent years. Oyster condition is largely dependent on reserves accumulated during summer feeding, and warmer temperatures stimulate higher productivity and feeding rates. The apparent difference between the values observed in our study versus Oliveira et al. (2006) is possibly explained by the cooling period KB has experienced over the past decade (Personal communication: Ray RaLonde 2009; unreferenced). In Oliveira et al. (2006), the objective was to determine the quality of oyster actually delivered to the market at given times. Thus, oysters were sampled at the wholesale level while in this project oysters were sampled at the site. In Oliveira et al. (2006), there may have been a selection of oysters that were sent for sale, which could have affected the quality. Periods of peak

reproductive development coincide with periods of greater food availability, improved meat quality, and accumulation of important energetic resources such as glycogen and lipids (Walne 1970, Gabbot & Stephenson 1974, Mann 1979, Hand & Nell 1999). Oysters increase in dry meat condition as they reach reproductive maturity (Walne 1970), and oysters sampled from KB are observed to have reached partial reproductive maturity (Fig. 3.2), which accounts for the higher condition recorded at KB prior to winter.

Kachemak Bay exhibits a characteristic late summer / early fall diatom bloom, a food source preferentially selected for and highly nutritious to Pacific oysters (Soudant et al. 1999, Whyte et al. 1990, Zieman 1990, Flores-Vergera et al. 2004, Cooney 2005, Ralonde et al. 2008). At KB in 2009, summer temperatures exceeded 10.5°C for a total of 56 days (Fig 3.2), which should promote growth and gametogenesis (Mann 1979). Conversely, winter conditions are harsh at KB and temperatures drop below 3°C for extended periods in 2006 to 2010 (Fig 3.2). Oyster feeding rates are limited by temperature, and may be nutritionally compromised at temperatures of between 3-10°C (Elsey 1933). Depletion of resources (Gabbot & Stephenson 1974, Walne & Mann 1975, Kang et al. 2000) contributes to the loss of solid tissue of oysters when winter temperatures are low (Mann 1979, Kang et al. 2000, Ren et al. 2003). This may account for the differences in CI^{HN} observed between sampling times.

3.5.1.2. Thorndyke Bay oysters

At TB, the control family of oysters had greater CI^{HN} in October of 2009, resulting from responses to feeding conditions and temperatures conducive to reproductive development (Fig. 3.2). Our values for CI^{HN} of oysters from TB were high, falling in the upper range of those of Whyte and Englar (1982), who reported values from 100 to 160 for on bottom and tray culture Pacific oysters from British Columbia (BC) in October in 1980. At TB, intertidal oysters exhibit an increase in CI^{HN} between sampling times (Tables 3.1 and 3.2), which is similar to patterns

observed by Whyte and Englar (1982) at lower latitudes of BC, where values for oysters from bottom and tray cultures both increased between October and June.

3.5.1.3. Site differences and MBP family comparisons

The two sites experienced a number of drivers affecting meat condition and quality such as accumulated nutritional reserves, partial reproductive development, and warmer temperatures that affect growth. These factors likely contribute to overall oyster quality at both sites. Temperature conditions were particularly similar between locations in October of 2009 (Fig 3.2), and corresponded to the similarities observed in the CI^{HN} of oysters sampled at KB and TB at the end of summer (Fig. 3.3 to 3.5). While oysters grown at TB experienced high levels of primary productivity and warmer summer conditions (NANOOs website 2011) than at KB, oysters grown at the higher latitude at KB experienced the benefit of long daylight hours and the associated increase in primary productivity. Oysters at TB were subject to twice daily exposure with the receding of tides, which results in a cessation of feeding and can lead to temporary anaerobic conditions and elevated body temperatures (Newell et al. 1978, David et al. 2005, Meisterzheim et al. 2009, Rafrafi & Uglow 2009). Oysters at KB were constantly submerged and had greater opportunity to feed during periods of high productivity but experienced colder water conditions, which can affect inhibit metabolic rates and feeding rates (Hochachka & Somero 1973, Brown & Hartwick 1988, Bougrier et al. 1998).

Oysters at KB had lower CI^{HN} than those at TB in June of 2010 (Fig. 3.3 to 3.5). The harsher winter conditions at KB compared to TB, combined with earlier onset of warmer and more productive conditions at TB, are a possible explanation of this difference. Furthermore, less challenging winters mean that when feeding recommences in the spring, oysters at TB had larger meats than in October of 2009 and a higher CI^{HN} than KB oysters. The differences in CI^{HN}

between sites in both October of 2009 and June of 2010 are consistent with differences in biochemical composition data.

Family 34 ranked consistently high with regards to CI^{HN} at KB in both October of 2009 and June of 2010, and this family CI^{HN} also ranked high at TB in June of 2010 (Table 3.1). The greater filling of the shell cavity with meat in family 34 oysters is a quality trait desirable to growers and preferred in the half-shell market (RaLonde & Painter 1993, Harrington 2005), which makes this family potentially valuable to breeders.

Shells of oysters from suspended culture tend to be thinner and more fragile than those of oysters raised on or near the bottom (Whyte & Englar 1982). To form shells oysters deposit layers of calcite-ostracum, which are frequently interrupted by more porous layers of "chalky" calcite crystals (Galtsoff 1964). Oysters submerged in the substrate deposit more impervious calcite-ostracum layers resulting in denser shells (Whyte & Englar 1982). Thicker shells of TB oysters may be an adaptive response to higher temperatures and associated availability of calcium carbonate, important in bivalve shell formation (Hochachka & Somero 1973, Vermeij 1978, Whyte & Englar 1982). Whyte & Englar (1982) propose that oysters submerged in or near the substrate in the intertidal zone generate thicker shells to compensate for abrasion and desiccation.

3.5.2. Biochemical composition

3.5.2.1. Proximate composition

3.5.2.1.1. Kachemak Bay oysters

Mean solids content of oysters sampled from KB (Table 3.4) for October ($15.2 \pm 0.5\%$) and June ($16.7 \pm 0.8\%$) appear similar to those reported by Martin et al. (2000), and lower than for KB oysters sampled by Oliveira et al. (2006) in the same months in 2003 (20.8%) and 2004 (17.5%). As with CI^{HN}, the solids content represents the meat content of the oyster. Observed patterns and changes in solids content are generally very similar to patterns of CI^{HN} (Tables 3.3

and 3.5). Harsher winters and cooler than average summers recently at KB mean that oysters were in poorer condition than those in the years 2003 and 2004, and also as compared with oysters in more temperate regions such as Ireland or WA. Consequently, KB oysters likely catabolized more of their nutritional reserves to maintain themselves over winters.

Ranges in protein content of oysters sampled from KB (Table 3.4) matched values observed by Whyte and Englar (1982) in British Columbia, and by Linehan et al. (1999) in Ireland. At KB, protein content of oysters was observed to decrease significantly between October of 2009 and June of 2010, and this is in agreement with Oliveira et al. (2006) results for oysters following over wintering at KB in 2003 to 2004 (Tables 3.4 and 3.5).

The mean glycogen content among oysters sampled from KB (Table 3.4) seems generally lower than that observed by Oliveira et al. (2006), Linehan et al. (1999), and also by Whyte & Englar (1982). Glycogen content at KB increased by 7.6% between October of 2009 and June of 2010, which is counter to the observations of Oliveira et al. (2006), who saw a slight decrease at KB from October of 2003 (48.0%) to June of 2004 (44.8%). In contrast, this increase agrees with the patterns observed by Whyte & Englar (1982) among oysters grown on or near the bottom in British Columbia, where an approximate 6% increase in glycogen between October of 1980 and June of 1981 was recorded. The likely explanation is that MBP oysters catabolized protein as an energy source to a greater degree than the control oysters (Tables 3.4 and 3.5). This is further supported by the observed negative linear correlation of protein as a function of glycogen in October of 2009 ($P > 0.05$, $R^2 = 0.13$), and June of 2010 ($P < 0.05$, $R^2 = 0.77$), and in the corresponding changes in protein and CI^{HN} recorded between October of 2009 and June of 2010 at KB (Tables 3.3 and 3.5).

Ash content at KB (Table 3.4) matched values typically observed by studies of this type (Whyte & Englar 1982, Whyte et al. 1990, Ruiz et al. 1992, Ren et al. 2003, Oliveira et al. 2006,

Dridi et al. 2007). There was an insignificant decrease in lipids between October of 2009 and June of 2010 at KB (Table 3.4 and 3.5), which is also consistent with the pattern observed by Oliveira et al. (2006). The ranges in lipid content across all families appear greater than the 6.1% and 3.8% dry weights observed by Oliveira et al. (2006) at KB in October of 2003 and June of 2004, respectively. However, the literature reports a wide range of lipid content for Pacific oysters (Whyte & Englar 1982). Our values fall within the range of lipid content reported for oysters in these studies but below the standard expected values of < 12% for oysters as suggested by Martin et al. (2000).

The biochemical composition and its observed changes between sampling times of oysters grown at high latitudes in this study show apparent unique patterns, particularly in response to seasonal changes when compared to studies of oysters sampled at lower latitudes. The sub-arctic climatic conditions differ considerably from those of the native range of the Pacific oyster, and this is likely responsible for the differences observed among Alaskan oysters. Oliveira et al. (2006) observed similar differences when comparing Alaska oysters to oysters grown in more temperate regions. Overall our observations suggest that selected MBP oysters generally possess the same biochemical qualities as wild and industry oysters, and no improvements were observed at KB resulting from selection. Oyster composition indicates that KB oysters meet or exceed quality standards seen elsewhere in the industry.

3.5.2.1.2. Thorndyke Bay oysters

At TB, oyster's solids content matched expected ranges for Pacific oysters reported by Martin et al. (2000). Values for solids content of oysters sampled from TB (Table 3.4) fell inside the ranges observed by Whyte & Englar (1982) in a seasonal study of oysters in British Columbia (16-22% solids). Mean solids content of MBP oysters at TB in October of 2009 (15.4%) were lower than those values observed by Oliveira et al. (2006) in October of 2003 (20.8%), but higher

in June of 2010 (19.2%) than in June of 2004 (17.5%). It is important to point out that Mann (1979), Whyte & Englar (1982), and Hand & Nell (1999) reported draining and blotting dry oysters preceding biochemical analysis. But in our study, as in Oliveira et al. (2006), we included cavity fluids when pooling and homogenizing oysters for analysis because it is an intrinsic portion of the product consumed.

Ranges of protein content of oysters from TB (Table 3.4) agreed with observations by Whyte & Englar (1982) in British Columbia oysters, Linehan et al. (1999) in Irish Pacific oysters, and Oliveira et al. (2006) in KB oysters. At TB, the protein content of oysters decreased between sampling times (Table 3.5), which is consistent with patterns of overwintering among oysters grown at lower latitudes in Ireland (Linehan et al. 1999), South Korea (Kang et al. 2000), and southern New Zealand (Ren et al. 2003). Seasonal studies in British Columbia (Whyte and Englar 1982) in Spain (Ruiz et al. 1992) did not show significant changes in protein content over winter and between the months of October and June.

As observed in KB oysters, the glycogen content among oysters sampled from TB (Table 3.4) was also generally lower but within the ranges observed by Oliveira et al. (2006), Linehan (1999) and Whyte & Englar (1982). The lack of significant differences observed in glycogen content of oysters sampled from TB between October of 2009 and June of 2010 (Table 3.5) is in agreement with patterns seen in studies at temperate latitudes (Kang et al. 2000, Ren et al. 2003, Dridi et al. 2007). These studies report the re-absorption of gametes when water temperatures cool as winter approaches, which is then followed by accumulation of glycogen with spring warming and associated increase in primary productivity, promoting more active feeding in preparation for reproductive development (Walne 1970, Ren et al. 2003, Dridi et al. 2007). In WA, temperatures never reached the 20°C required for spawning (Fig. 3.2). In this case, oyster

glycogen reserves remained stable because their depletion was likely counterbalanced by good feeding conditions maintaining reserves.

The ash content was low at TB (Table 3.4), falling within expected ranges of Pacific oysters (Martin et al. 2000), and matched those values between 1 and 2% observed by most studies of this type (Whyte & Englar 1982, Ren et al. 2003, Oliveira et al. 2006). At TB, observed values for lipid content of oysters fell in the middle of the ranges in these studies, but below the standard expected values for oysters of < 5% as determined by Martin et al. (2000). Observed lipid content among TB oysters in our study appear slightly higher than values reported in Irish Pacific oysters (~8% dry weight) by Linehan et al. (1999), but within the range (5.8 to 15.3%) seen by Costa-Muniz et al. in oysters grown in Brazil (1986). There was a small increase in lipid content of oysters sampled in October of 2009 as it compares to values in June of 2010 oysters, although it was not significant ($P > 0.05$, Tables 3.4 and 3.7). Linehan et al. (1999) and Dridi et al. (2007) also recorded small changes in lipids (~1-2% dry weight) between similar periods of the year. This increase in lipid may be attributed to precursory preparation for reproductive development observed in oysters in the spring (Dridi et al. 2007).

It appears that the oysters grown at TB in this study show similar patterns of biochemical composition to those oysters sampled in studies typically seen at similar latitudes. The changes in composition of TB oysters between sampling times followed seasonal cycles and changes expected in oysters grown in temperate regions, which was in agreement with the patterns observed in the literature.

3.5.2.1.3. Site differences and MBP family comparisons

At KB in October of 2009, high CI^{HN} and relatively greater solids content (Tables 3.1 and 3.5) are indicative of meat growth (Walne & Mann 1975). This good condition is likely a function of the late summer diatom bloom characteristic of KB (KBRR NERRS System Wide Monitoring

Program 2010). In June of 2010, the later onset of warming water conditions at KB relative to TB, caused by summer glacial runoff reducing water temperature and salinity in the immediate area, is likely inhibiting growth at this time (Bernard 1983, Brown & Hartwick 1988, Quayle 1988, Field & Walker 2003). Low temperatures below 10°C inhibit feeding in Pacific oysters and feeding completely stops below 3°C with valve closure (Elsey 1933). At KB, temperatures of below 3°C were observed for a cumulative 340 days between 2006 and 2010. At low temperature, oyster growth is suppressed because feeding and metabolic processes are reduced (Hochachka & Somero 1973, Gricourt et al. 2003). Low temperatures, leading to valve closure for prolonged periods at KB in winter, or during exposure at low tide at TB, may have resulted in anaerobic metabolism (Rafrafi & Uglow 2009). Bivalve molluscs will typically catabolize carbohydrates and amino acids when out of the water or under anaerobic conditions (Newell 1978, David et al. 2005, Rafrafi & Uglow 2009).

At KB, similar changes in protein content of the oysters at both sites in October of 2009 matched changes in CI^{HN} (Tables 3.1and 3.4) and lipid content (Tables 3.4 and 3.6). This supports our observation that oysters grown at both locations were in 'excellent' condition (Fig. 3.3A), and that oysters had been feeding and were growing during summer months prior to sampling. Walne (1970) reported dry meat condition index increasing with latitude, but the author did not study latitudes as far north as AK. The CI^{HN} index is closely tied to meat weight (Equation 3.1), the largest constituent of which is protein (Table 3.4). Protein is also a major component of oyster tissue and gametes (Walne & Mann 1975, Whyte & Englar 1982, Ren et al. 2003).

Glycogen is used as an energetic reserve for egg development (Hochachka & Somero 1973), and it is also important in maintaining essential metabolic processes and growth (Mann 1979, Whyte & Englar 1982, Whyte et al. 1990). Elevated glycogen levels can indicate a build-up of reserves for use in reproductive development (Walne 1970, Oliveira et al. 2006, Dridi et al.

2007). Typically, summer warming periods see increases in protein and lipids at the expense of glycogen as reproductive development begins in Pacific oysters (Berthelin et al. 1990, De La Parra et al. 2005), this pattern was also noted oysters at TB (Tables 3.4 and 3.5). Of interest is the significant negative linear correlation between protein and glycogen content of oysters grown at TB in October of 2009 ($R^2 = 0.77$), and at both sites in June of 2010 ($R^2 = 0.61$ and 0.95 at KB and TB, respectively). Notably, storage of glycogen at KB over winter takes place at the expense of protein resources (Tables 3.4 and 3.5). The higher glycogen observed at KB relative to TB suggests less reproductive development, as glycogen is used to provide the early growth season energy for the synthesis of gametic protein and lipid (Mann 1979, Ruiz et al. 1992, Linehan et al. 1999, Ren et al. 2003, Dridi et al. 2007).

The higher lipid content in control oysters in June of 2010 at TB is likely due to gametogenesis. Fluctuations in lipid content of Pacific oysters frequently have a strong significant positive correlation with glycogen (Walne & Mann 1975), and a positive correlation between these variables was observed at TB ($P < 0.05$; $R^2 > 0.22$). However, no significant correlation was observed between glycogen and lipids at KB at either sampling time ($P > 0.05$). Furthermore, Walne & Mann (1975) also reported a strong negative correlation between lipid and protein content. In our study a significant positive correlation between lipid and protein content was observed at both KB and TB in October of 2009 and June of 2010 ($P < 0.05$; $R^2 \geq 0.45$). MBP Cohort 20 family 28 ranked consistently high in terms of solid content, glycogen, and lipid content at KB in both October of 2009 and June of 2010. Of note is the fact that this family also ranked high in amount of total gonad area occupying the visceral mass. All of these factors combined contribute to explaining the success of this MBP family when grown at KB, and as such may warrant future attention of growers and breeding programs. Cohort 20 families 44 and

34 also ranked consistently high with regards to protein and glycogen contents in both October of 2009 and June of 2010 (Table 3.4).

Selection for improved yields, growth, and survival by MBP has not had a detrimental effect upon nutritional quality of oysters grown in either site studied. Oysters grown at the high latitudes of KB are benefiting from cooler temperatures and inhibited reproductive development. Consequently, oysters at KB possess an increased ability to conserve glycogen throughout the summer. The higher protein content at TB is an indicator of elevated reproductive development and gamete generation when compared to KB at the end of summer in October of 2009, and is a function of more accumulated days above 10.5°C (Mann 1979), as at TB in June of 2010 (NANOOS website 2011; Fig. 3.2). The significant observed difference in protein content at KB after winter when compared to TB may also be an indicator that the oysters were starving during periods of temperatures below 3°C (Elsey 1933, Li et al. 2009) (Fig. 3.2). At KB, protein utilization to conserve glycogen over winter comes at a potential cost to oyster health, unlike oysters grown at the lower latitude of TB. In terms of oyster quality, high levels of glycogen are desirable to consumers and have premium market appeal (Conte et al. 1997, Harrington 2005). Observations of proximate composition suggest that selected MBP oysters generally do not deviate from the biochemical characteristics from oyster stocks as exemplified by the control family in this study, with the exception of greater levels of lipids at KB and protein at TB in June of 2010 in the control family.

3.5.2.2. Fatty acid composition

3.5.2.2.1. Kachemak Bay oysters

The distribution of fatty acid classes determined in KB oysters in June of 2010 (Table 3.6) agreed with values observed by Oliveira et al. (2006). The PUFA content of KB oysters determined in our study were comparable to the 51.9% determined by Oliveira et al. (2006), but

higher than the values for Irish Pacific oysters of 57.3% (Linehan et al. 1999). The omega-3 levels constituting the majority of the PUFA (47.2%), are similar to those reported by Oliveira et al. (2006). However, the omega-6 content was higher in oysters from KB in June of 2004 (4.7%) than in our study in June of 2010. Of the omega-3 FAs, EPA was higher than values reported for KB oysters in 2003 (21.2%), but in the range of values observed in Japanese Pacific oysters (22.4%; Jeong et al. 1990).

The DHA content appears similar to KB oysters in 2004 (16.5%), but above ranges of Irish Pacific oysters (10.3 to 15.5%) reported by Linehan et al. (1999). The DHA content was also higher at KB in June of 2010 than in Japanese Pacific oysters cultured in Hiroshima Bay at ~ 34° latitude (11.0%; Jeong et al. 1990). Values of SFA (23.75%) and MUFA (19.5%) content of oysters from KB in June 2003 agreed with Oliveira et al. (2006), and with Irish Pacific oysters (25.1% SFA; 17.5% MUFA) as reported by Linehan et al. (1999). Palmitic acid, the most abundant SFA, was very similar to Irish Pacific oysters (16.0%) peaking in the summer and showing little annual variation (3.1%). In summary, the levels of PUFA at KB were higher than reported for oysters from lower latitudes, supporting observations that lower environmental temperatures promote increased levels of these fatty acids in marine organisms (Valentine & Valentine 2010).

3.5.2.2.2. Thorndyke Bay oysters

At the lower latitude of TB, the distribution of FA classes was also similar to those seen by Oliveira et al. (2006) at KB. The levels of PUFA at TB (Table 3.6) were slightly lower than in Irish Pacific oysters (Linehan et al. 1999). Both omega-3 and omega-6 FA were lower at TB than at KB in 2003 (Oliveira et al. 2006), with omega-3 values being closer to values observed by Jeong et al. (1990) in Japanese Pacific oysters (40.0%). The EPA content was also closer to the 21.2% observed in Japanese Pacific oysters (Jeong et al. 1990). The DHA content was also lower

than the 16.2% determined for KB oysters in 2003 (Oliveira et al. 2006), but fell within the mid-range for Irish Pacific oysters, where DHA decreased to 11.0% as June approached (Linehan et al. 1999), and of Spanish Pacific oysters in June (Ruiz et al. 1992). Values for the SFA, particularly palmitic acid matched well with KB oysters in 2004 (Oliveira et al. 2006), and with values for Irish Pacific oysters in 1981 (Linehan et al. 1999). The FA composition of oysters grown at TB in this study followed similar patterns to oysters grown at lower latitudes, closer to the native range of the Pacific oyster.

3.5.2.2.3. Site differences and MBP family comparisons

High temperature has been correlated with higher levels of SFA and lower levels of PUFA in Pacific oysters (Flores-Vergara et al. 2004). Oysters grown by suspended culture at KB exhibit significantly greater levels of PUFA than those oysters from TB (Tables 3.6 and 3.8). The levels of SFA and MUFA were significantly higher at the lower latitude of TB. This is likely attributed to relatively higher average seasonal temperatures (NANOOS website 2011; Fig. 3.2) and exposure to solar heating during low tides. MBP Cohort 20 oysters at KB possessed relatively greater abundances of long chain omega-3 fatty acids, such as DHA, when compared to TB. Long chain omega-3 fatty acids (specifically 20:5ω3 and 22:6ω3) are essential nutrients necessary for growth (Waldock & Holland 1984), as well as being constituents of cellular membranes and enhancing membrane fluidity and cold tolerance of marine organisms (Hall et al. 2010). The DHA molecule is predominantly formed in photosynthesizing cells at low temperatures, and is more abundant at higher latitudes in a number of marine species (Valentine & Valentine 2010). PUFA are also energetically and structurally important in gametes (Gabbott & Stephenson 1974, Ruiz et al. 1992, De La Parra et al. 2005, Dridi et al. 2007). Oysters are able to synthesize long chained fatty acids from shorter chained fatty acids, but largely rely on their phytoplankton diet

for supplies of EPA and DHA (Gabbott & Stephenson 1974, Waldock & Holland 1984, Dridi et al. 2007).

In summary, significantly cooler temperatures at KB compared to TB (Fig. 3.2) were responsible for elevated levels of PUFA particularly the long chain omega-3 fatty acids, the most abundant of which was DHA. Family 44 appeared to accumulate PUFAs more than other families and may warrant attention in future breeding programs. In terms of cardio and neural health benefits associated with PUFA (Sargent 1997, Linehan et al. 1999, Mattes 2005, Ruxton et al. 2005), consumers of KB oysters may benefit from the higher levels of EPA and DHA found in the product as it compares to oysters grown at lower latitudes, and this attribute might be considered as a valuable trademark for growers in this area.

3.5.3. Reproductive condition

3.5.3.1. Kachemak Bay oysters

Temperatures at KB exceeded 10.5°C for 56 days, but temperatures never exceeded 12.6°C in 2009 (Fig. 3.2). Enough thermal units accumulated to initiate some gametogenesis, and reproductive development was more advanced at the end of the summer than after winter (Fig. 3.2 and Table 3.9) (Mann 1979, Dridi et al. 2007).

3.5.3.2. Thorndyke Bay oysters

Temperatures at the Seattle monitoring station exceeded 10.5°C for 174 days in 2009 (Fig. 3.2), which was less than the threshold necessary for oysters to reach reproductive maturity in Mann's study (1979). Temperatures from oceanographic cruise data collected offshore at TB (NANOOS website, 2011) were ~12°C in October to ~13°C in June in years where sampling took place, and continuous monitoring buoys at nearby Dabob Bay reported peak surface temperatures of ~19-20°C, but only for very short periods of time. Reabsorption of gametes may have been

occurring at TB by mid October of 2009 and sufficient thermal accumulation may have begun by June of 2010 (Mann 1979, Steele & Mulcahy 1999), explaining the lack of significant differences in reproductive development at TB between sampling times.

3.5.3.3. Site differences and MBP family comparisons

The degree of gametogenesis and reproductive development is one of the dominant influences in the life history and biochemistry of the Pacific oyster and can vary significantly between locations (Walne 1970). The data shows that the level of reproductive development was significantly less at KB compared to TB in both October of 2009 and June of 2010 (Table 3.9 and Fig. 3.2). At KB, October oysters were more mature. This is likely a function of the temperature regimes experienced by these oysters and follows similar patterns of reproductive development observed in oysters at varying latitudes and temperatures (Gabbott & Stephenson 1974, Mann 1979, Kang et al. 2000, Dridi et al. 2007). At the end of the summer in 2009, temperatures at the KB site had been in excess of 10.5°C for a total of 56 days and rarely exceeded 12°C. In previous years, including 2009, temperatures had yet to exceed 10.5°C by June. Temperatures were still as low as 8.3°C by May 10 of 2010 at KB. Before June at KB little to no gametogenesis would have occurred, as the temperature threshold of 10.5°C for this process in Pacific oysters would likely not have been reached. This is supported by histological analysis; results revealed that KB oysters at this time only had reached stage 2 development (developing gametes), whereas at TB oysters were observed at the more advanced stage 3 (ripe gametes), additional evidence that levels of gametogenesis varied between locations. The stages of development match values observed at corresponding temperature regimes observed by Mann (1979). Food consumption is also an influential factor in regard to reproductive development. Oyster feeding rates are limited by temperature and are seriously, if not completely, compromised at temperatures of between 3-10°C

(Elsey 1933). Depressed feeding or starvation prior to reproductive activity is known to negatively affect gametogenesis (Chavez-Villalba et al. 2007).

The difference in the level of reproductive development between sites is a function of latitudinal and environmental differences, which vary over temporal scales. Of note is the lack of differences in reproductive development between October of 2009 and June of 2010 at TB (Table 3.9 and Fig. 3.2). Although overwintering should suppress gamete development, warming spring weather in May leading up to June (Fig 3.2) may have initiated early development. The month of October is also a period when oysters are in a recovery stage post-spawning or post-gametogenesis (Dridi et al. 2007).

MBP Cohort 20 family 28 ranked highest in terms of reproductive development and it was able to manifest a relatively higher degree of gametogenesis under adverse cool conditions not typically conducive to reproductive development in Pacific oysters (Mann 1979). In October of 2009 and June of 2010 at KB, family 20 was ranked lowest in terms of reproductive development at KB.

3.6. Conclusions

Meat quality is rated very highly by consumers of oysters (Conte et al. 1997, Harrington 2005). The quality of the MBP oysters varied by region and by season. Cold water temperatures and low salinities from local glacial runoff at KB present challenges to oyster growers, creating environmental conditions that slow growth and suppress feeding activity during mid-winter (January to March). Colder water temperatures and good feeding conditions late into the summer at KB convey an advantage to a number of quality traits, and to a lesser degree at TB. Limited reproductive development means that oysters at KB do not utilize much of their glycogen and lipid reserves, which are typically lost during spawning events at lower latitudes in mid and late summer.

The lower than average winter temperatures leading to reduced meat weights, slow growth, and observed sacrifice of protein at the expense of glycogen may explain low winter survival of oysters at KB. At TB, oysters grown intertidally and subject to the mechanical action of waves and tides, become deep-cupped oysters with strong shells and good meat fill. Consumers like an oyster with a deep cup (Brake at al. 2003) that is full of meat (Hand & Nell 1999), while thick-shell oysters are less susceptible to predatory dangers and the threat of changing climatic conditions.

The reproductive cycle of oysters is a determining factor in the accumulation and depletion of biochemical components of oyster tissues. Of the biochemical parameters of interest, glycogen is important in conveying a sweet taste, and lipids impart a creamy feeling in the mouth that many consumers find desirable (Rolls et al. 1999). Cool temperatures inhibit reproductive development among oysters, as we observed among oysters from KB when compared to TB. This in turn led to oysters from the higher latitudes of AK maintaining relatively greater glycogen levels than oysters from the lower latitude of TB. Furthermore, at KB, colder temperatures have apparently led to relatively higher concentrations of DHA, a molecule important in maintaining cell membrane fluidity at low temperatures (Valentine & Valentine 2010). Notably, DHA and other PUFA are known to be important in cardio and neural health, which is beneficial to consumers of oysters (Sargent 1997, Linehan et al. 1999, Mattes 2005, Ruxton et al. 2005). Unique quality attributes such as sweetness from elevated glycogen, or nutritional advantages of PUFA such as DHA and EPA inherent in oysters grown using suspended culture at KB may be trademarked and marketed by Alaska growers to vendors. Supporting a targeted program for further broodstock is a program worth pursuing in Alaska.

3.7. References

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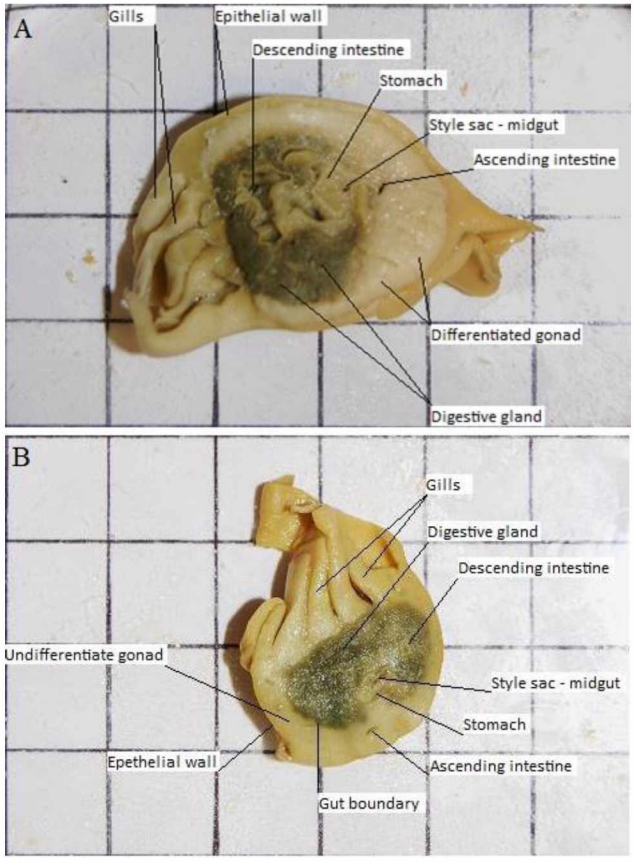

Figure 3.1. Transverse section of *C. gigas* in the region of the digestive gland. (A) Differentiated gonad. (B) Undifferentiated gonad.

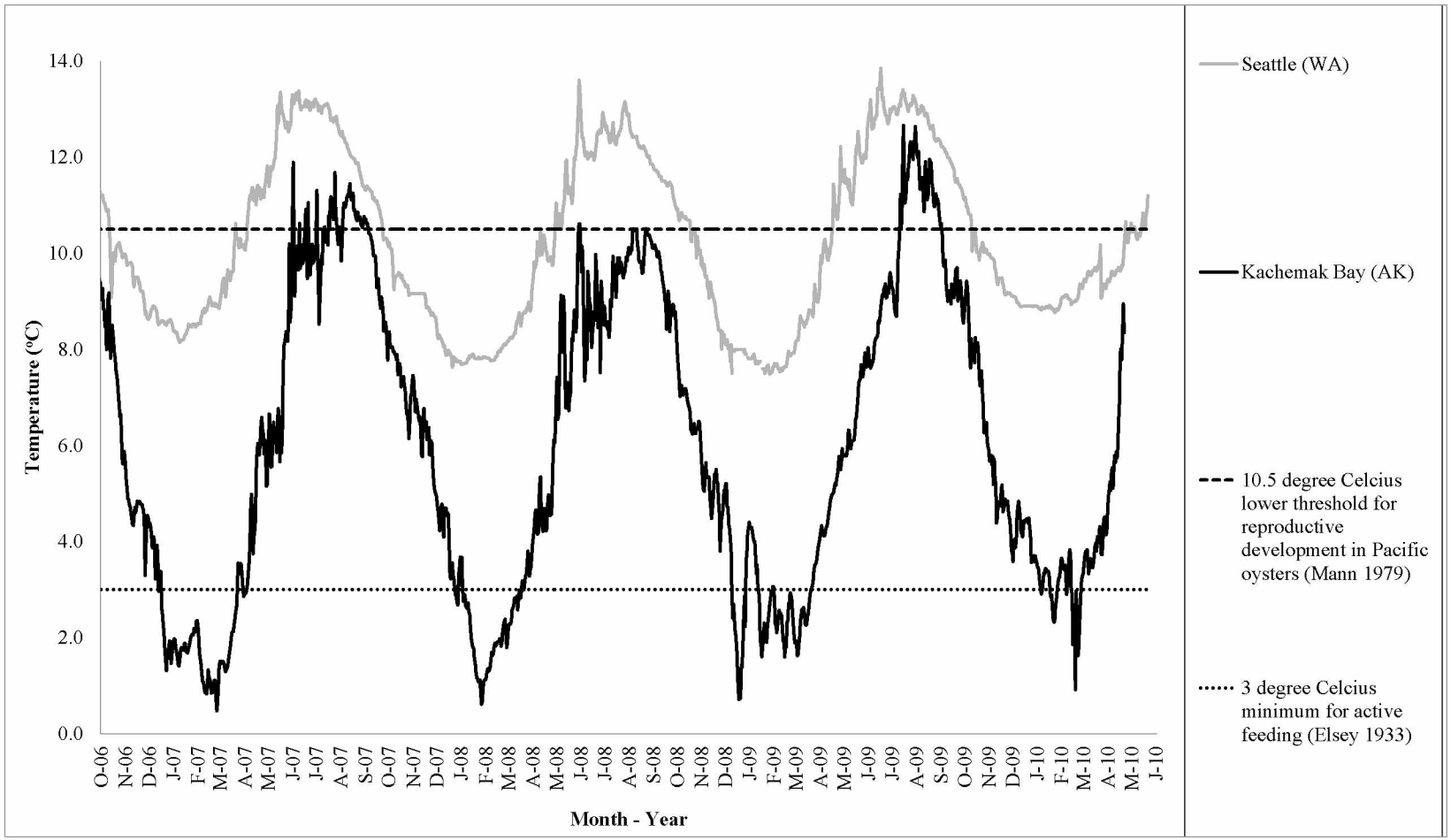

Figure 3.2. Seasonal variations in daily temperature (°C) at KB, AK. Peterson Bay, KB, AK oyster growout site (Source: NERRS Kachemak Bay, daily temperature records) and Seattle, WA (Source: NOAA Tides and Currents, Seattle Meteorological Conditions website, daily temperature records) 2005 to 2010.

Table 3.1. Meat weight, cavity volume, and meat condition of oysters sampled from KB, AK, and TB, WA, in October of 2009.

Family #	28	24	46	20	34	44	21	0	Mean
Location	**Kachemak Bay (AK) - October 2009**								
Meat weight (g)	11.5^{ab}	11.2^{ab}	15.8^{a}	11.1^{ab}	12.4^{ab}	10.5^{ab}	13.9^{ab}	7.5^{ab}	11.8
	(0.9)	(1.9)	(2.2)	(2.0)	(1.8)	(0.9)	(1.8)	(0.8)	(1.5)
Cavity volume (g)	13.3^{a}	12.1^{a}	17.1^{a}	14.2^{a}	16.7^{a}	11.5^{a}	15.6^{a}	7.9^{a}	13.6
	(3.8)	(2.0)	(2.3)	(3.1)	(3.4)	(1.0)	(2.0)	(1.0)	(2.3)
CI^{HN} - Meat fill index	168.5^{a}	147.1^{a}	137.6^{a}	129.1^{a}	171.4^{a}	141.2^{a}	148.9^{a}	184.1^{a}	153.5
	(9.7)	(12.0)	(8.9)	(16.3)	(11.2)	(10.5)	(12.5)	(25.2)	(13.3)
Location	**Thorndyke Bay (WA) - October 2009**								
Meat weight (g)	67.0^{a}	59.2^{a}	69.3^{a}	60.1^{a}	68.3^{a}	55.3^{a}	69.0^{a}	49.8^{a}	62.2
	(3.3)	(5.9)	(4.6)	(5.6)	(5.8)	(3.9)	(6.2)	(4.7)	(5.0)
Cavity volume (g)	66.8^{a}	59.3^{a}	70.5^{a}	60.4^{a}	68.2^{a}	55.4^{a}	68.1^{a}	48.4^{a}	62.1
	(3.1)	(5.7)	(4.5)	(5.2)	(5.6)	(3.5)	(6.0)	(4.6)	(4.8)
CI^{HN} - Meat fill index	122.9^{a}	128.7^{a}	117.1^{a}	120.3^{a}	147.7^{a}	126.3^{a}	127.2^{a}	143.1^{a}	129.2
	(8.5)	(9.2)	(4.4)	(6.8)	(10.1)	(5.5)	(9.2)	(7.9)	(7.7)

Standard deviation of the mean (SD). Different superscript letters represent significant differences between families for each growth and biometric parameter (ANOVA: Tukey's Test, $n = 3$, 15 d.f., significant $P < 0.05$) differences were only tested for within sites and site interaction was not tested for families.

Table 3.2. Meat weight, cavity volume, and meat condition of MBP oysters sampled from KB, AK, and TB, WA, in June of 2010.

Family #	28	24	46	20	34	44	21	0	Mean
Location	**Kachemak Bay (AK) – June 2010**								
Meat weight (g)	15.1[a]	13.2[a]	14.5[a]	13.2[a]	12.9[a]	13.8[a]	10.1[a]	10.7[a]	12.9
	(5.2)	(5.4)	(4.9)	(4.4)	(7.5)	(4.6)	(3.9)	(7.6)	(5.4)
Cavity volume (g)	17.0[ab]	14.4[ab]	18.8[a]	14.3[ab]	12.8[ab]	14.5[ab]	12.1[ab]	11.2[b]	14.4
	(5.7)	(5.9)	(4.9)	(4.5)	(7.5)	(4.9)	(5.8)	(8.1)	(5.9)
CI^{HN} - Meat fill index	122.9[a]	128.7[a]	117.1[a]	120.3[a]	147.7[a]	126.3[a]	127.2[a]	143.1[a]	129.2
	(8.5)	(9.2)	(4.4)	(6.8)	(10.1)	(5.5)	(9.2)	(7.9)	(7.7)
Location	**Thorndyke Bay (WA) – June 2010**								
Meat weight (g)	53.4[ab]	56.5[a]	54.9[ab]	59.4[a]	56.7[a]	61.7[a]	48.2[ab]	37.4[b]	53.5
	(11.8)	(13.7)	(16.8)	(15.5)	(13.0)	(14.2)	(11.8)	(10.6)	(13.4)
Cavity volume (g)	54.3[a]	58.3[ab]	55.8[ab]	63.4[ab]	58.2[ab]	63.3[ab]	49.2[ab]	40.1[b]	55.3
	(12.2)	(14.1)	(16.5)	(16.0)	(13.1)	(14.5)	(12.9)	(11.1)	(13.8)
CI^{HN} - Meat fill index	176.8[a]	186.6[ab]	204.4[ab]	167.7[ab]	192.3[ab]	158.3[ab]	186.4[ab]	179.2[b]	181.5
	(9.4)	(7.3)	(9.8)	(10.1)	(5.1)	(3.6)	(11.6)	(10.0)	(8.3)

Standard deviation of the mean (SD). Different superscript letters represent significant differences between families for each growth and biometric parameter (ANOVA: Tukey's Test, $n = 3$, 15 d.f., significant $P < 0.05$) differences were only tested for within sites and site interaction was not tested for families.

Table 3.3. Comparisons of condition indices of MBP oysters between top seven MBP families (S: selected at KB) versus a control family (C), between sites, and between seasons.

Interaction	**Site * season * control**				**Site**		**Season**	
Location / Sample time	**KB Oct - 09**	**TB Oct - 09**	**KB June - 10**	**TB June - 10**	**KB**	**TB**	**Oct - 09**	**Jun - 10**
Comparison	**Control vs. 7 selected MBP families**				**Oct - 09 vs. Jun - 10**		**KB vs. TB**	
CI^{HN} Meat fill index	C > S (0.0467^{A})	C > S (0.0081^{A})	C = S (0.1024^{A})	C = S (0.8352^{A})	Oct 09 > June 10 (0.0000^{A})	Oct 09 < June 10 (0.0000^{A})	KB = TB (0.4826^{A})	KB < TB (0.0000^{A})
Meat weight (g)	S > C (0.0069^{A})	S > C (0.0118^{A})	S = C (0.1169^{A})	S > C (0.0003^{A})	Oct 09 = June10 (0.1802^{A})	Oct 09 < June10 (0.0018^{A})	KB < TB (0.0000^{A})	KB < TB (0.0000^{A})
Cavity volume (g)	S > C (0.0088^{A})	S > C (0.0088^{A})	S = C (0.0659^{A})	S > C (0.0076^{A})	Oct 09 = June10 (0.5892^{A})	Oct 09 = June10 (0.2907^{A})	KB < TB (0.0000^{A})	KB < TB (0.0000^{A})

Kachemak Bay (KB). Thorndyke Bay (TB). S: MBP families from selected broodstock. C: MBP controls from unselected broodstock. Statistical differences ($P < 0.05$). KB = TB no significant difference detected between oysters grown at KB and TB. KB > TB (P-value) parameter is significantly greater in oysters grown at KB than at TB. Statistical test used: $\#.\#\#\#\#^{A}$ = ANOVA and $\#.\#\#\#\#^{KW}$ = Kruskal-Wallis test.

Table 3.4. Proximate composition for MBP families and the unselected control family of *C. gigas* sampled from KB and TB.

Family #	28	24	46	20	34	44	21	0	Mean
Location Sample time	**Kachemak Bay (AK) - October 2009**								
Solids (% wet wt.)	20.3[a]	18.3[ab]	15.4[c]	16.7[bc]	15.3[c]	16.1[bc]	15.4[c]	15.2[c]	16.6
	(0.4)	(0.4)	(0.3)	(0.6)	(0.5)	(0.3)	(1.0)	(0.8)	(0.5)
Ash (% dry wt.)	10.1[b]	13.2[ab]	14.2[ab]	14.9[ab]	15.3[a]	13.7[ab]	12.5[ab]	14.3[ab]	13.5
	(0.6)	(1.0)	(1.2)	(1.8)	(0.7)	(1.2)	(0.2)	(0.5)	(0.9)
Protein (% dry wt.)	51.7[a]	49.2[a]	49.8[a]	49.9[a]	51.7[a]	43.8[a]	50.5[a]	53.9[a]	50.1
	(1.1)	(0.9)	(1.3)	(2.0)	(0.5)	(5.3)	(1.2)	(0.7)	(1.7)
Lipids (% dry wt.)	8.5[a]	8.6[a]	9.4[a]	12.7[a]	7.0[a]	14.6[a]	8.7[a]	5.9[a]	9.4
	(1.9)	(0.4)	(2.5)	(5.2)	(1.1)	(7.6)	(0.7)	(0.4)	(2.5)
Glycogen (% dry wt.)	29.7[a]	29.1[a]	26.7[a]	22.5[a]	26.1[a]	27.9[a]	28.3[a]	26.0[a]	27.0
	(0.4)	(1.1)	(2.7)	(2.3)	(1.7)	(1.4)	(0.6)	(0.6)	(1.4)
Location Sample time	**Kachemak Bay (AK) – June 2010**								
Solids (% wet wt.)	17.9[ab]	12.2[ab]	13.9[ab]	12.6[a]	15.4[b]	15.2[ab]	10.3[ab]	13.6 [a]	13.9
	(3.0)	(0.5)	(1.0)	(1.0)	(0.5)	(0.5)	(2.4)	(1.3)	(1.3)
Ash (% dry wt.)	11.7[a]	14.5[a]	12.6[a]	13.6[a]	8.3[a]	13.1[a]	22.3[a]	13.6[a]	13.7
	(1.1)	(0.7)	(0.4)	(1.7)	(1.3)	(0.7)	(8.3)	(1.7)	(2.0)
Protein (% dry wt.)	37.2[a]	44.3[a]	43.5[a]	45.8[a]	41.0[a]	39.4[a]	41.7[a]	47.9[a]	42.6
	(1.2)	(0.4)	(2.6)	(1.1)	(2.7)	(1.2)	(3.0)	(3.0)	(1.9)
Lipids (% dry wt.)	10.7[a]	8.2[a]	9.0[a]	8.4[a]	9.6[a]	10.5[a]	6.8[a]	9.3[a]	9.1
	(0.5)	(0.6)	(0.3)	(0.6)	(0.7)	(0.2)	(1.2)	(1.5)	(0.7)
Glycogen (% dry wt.)	40.4[a]	33.1[a]	34.9[a]	32.1[a]	41.1[a]	37.1[a]	29.2[a]	29.2[a]	34.6[a]
	(2.4)	(1.0)	(2.6)	(1.9)	(1.9)	(2.1)	(4.3)	(4.2)	(2.6)
Location Sample time	**Thorndyke Bay (WA) - October 2009**								
Solids (% wet wt.)	14.3[a]	15.2[ab]	15.6[ab]	16.9[ab]	13.8[ab]	14.2[ab]	15.7[b]	16.7[ab]	15.4
	(0.3)	(0.2)	(0.7)	(1.0)	(0.5)	(0.4)	(0.3)	(0.8)	(0.6)
Ash (% dry wt.)	18.5[a]	15.8[a]	16.2[a]	14.2[a]	16.6[a]	15.9[a]	13.2[a]	12.9[a]	15.4
	(0.8)	(1.2)	(1.3)	(1.7)	(0.9)	(0.3)	(2.3)	(1.0)	(1.2)
Protein (% dry wt.)	54.6[b]	55.5[ab]	48.7[ab]	52.0[ab]	46.9[ab]	52.6[ab]	50.7[ab]	51.1[a]	51.5
	(2.5)	(4.4)	(3.3)	(2.8)	(1.0)	(2.5)	(2.8)	(2.8)	(2.7)
Lipids (% dry wt.)	8.1[a]	6.7[a]	8.6[a]	7.7[a]	8.7[a]	8.6[a]	11.5[a]	8.7[a]	8.6
	(0.6)	(1.2)	(1.8)	(1.3)	(1.0)	(0.3)	(0.4)	(0.6)	(0.9)
Glycogen (% dry wt.)	18.8[a]	22.1[a]	26.5[a]	26.1[a]	27.8[a]	22.9[a]	24.6[a]	27.3[a]	24.5
	(1.8)	(2.3)	(2.6)	(0.5)	(1.1)	(2.6)	(1.5)	(3.3)	(2.0)

Table 3.4. continued.

Family #	28	24	46	20	34	44	21	0	Mean
Location Sample time	**Thorndyke Bay (WA) – June 2010**								
Solids (% wet wt.)	18.8^{a}	20.3^{a}	20.8^{a}	17.0^{a}	20.1^{a}	16.9^{a}	18.9^{a}	21.1^{a}	19.2^{a}
	(0.72)	(1.03)	(1.10)	(1.39)	(0.59)	(0.36)	(0.79)	(0.81)	(0.85)
Ash (% dry wt.)	9.8^{abc}	8.9^{bc}	9.1^{bc}	10.1^{ab}	8.8^{c}	10.3^{a}	9.4^{abc}	9.1^{bc}	9.4
	(0.2)	(0.4)	(0.3)	(0.2)	(0.2)	(0.1)	(0.3)	(0.2)	(0.2)
Protein (% dry wt.)	61.2^{abc}	55.4^{bc}	56.5^{bc}	63.0^{ab}	54.8^{bc}	64.5^{a}	58.6^{abc}	56.6^{bc}	58.8
	(1.1)	(2.6)	(1.6)	(1.0)	(1.0)	(0.7)	(2.1)	(1.5)	(1.4)
Lipids (% dry wt.)	9.7^{ab}	9.0^{ab}	8.7^{b}	7.2^{b}	10.0^{ab}	7.2^{b}	9.5^{ab}	11.7^{a}	9.1
	(0.2)	(0.4)	(0.8)	(0.5)	(0.6)	(0.5)	(0.9)	(0.6)	(0.6)
Glycogen (% dry wt.)	19.4^{ab}	26.8^{a}	25.8^{ab}	19.8^{ab}	26.5^{a}	18.0^{b}	22.6^{ab}	22.7^{ab}	22.7
	(1.4)	(3.3)	(1.3)	(0.8)	(0.6)	(0.6)	(1.5)	(1.3)	(1.4)

Standard deviation of the mean (SD). Different superscript letters represent significant differences between families for each proximate component parameter (Tukey's Test, $n = 3$, 15 d.f., significant $P < 0.05$), differences were only tested for within sites and site interaction was not tested for families. Percent dry wt. = percent of solid component. Percent oisture (not presented) = 100 – Solids.

Table 3.5. Comparisons of proximate composition of MBP oysters between top seven MBP selected families (S; selected at KB) versus a control family (C), between sites, and between sampling times.

Interaction	**Site * season * control**				**Site**		**Season**	
Location Sample time	**KB Oct - 09**	**TB Oct - 09**	**KB June - 10**	**TB June - 10**	**KB**	**TB**	**Oct - 09**	**Jun - 10**
Comparison	**Control vs. 7 selected MBP families**				**Oct - 09 vs. Jun - 10**		**KB vs. TB**	
Solids (% wet wt.)	C = S (0.1828 ^{A})	C = S (0.0658 ^{A})	C = S (0.8557 ^{A})	C = S (0.0775 ^{A})	Oct 09 > June 10 (0.0008 ^{A})	Oct 09 < June 10 (0.0000 ^{A})	KB > TB (0.0097 ^{A})	KB < TB (0.0000 ^{A})
Ash (% dry wt.)	C = S (0.5244 ^{A})	C = S (0.0732 ^{A})	C = S (0.9600 ^{A})	C = S (0.3353 ^{A})	Oct 09 = June 10 (0.4705 ^{KW})	Oct 09 > June 10 (0.0000 ^{A})	KB < TB (0.0087 ^{A})	KB > TB (0.0011 ^{KW})
Protein (% dry wt.)	C = S (0.0830 ^{A})	C = S (0.4760 ^{A})	C > S (0.0251 ^{A})	C = S (0.3280 ^{A})	Oct 09 > June 10 (0.0000 ^{KW})	Oct 09 < June 10 (0.0000 ^{A})	KB = TB (0.6353 ^{KW})	KB < TB (0.0000 ^{A})
Lipids (% dry wt.)	C = S (0.2666 ^{A})	C = S (0.9021 ^{A})	C = S (0.7681 ^{A})	C > S (0.0021 ^{A})	Oct 09 = June 10 (0.0797 ^{KW})	Oct 09 = June 10 (0.3342 ^{A})	KB = TB (0.3865 ^{KW})	KB = TB (0.9282 ^{A})
Glycogen (% dry wt.)	C = S (0.5394 ^{A})	C = S (0.2331 ^{A})	C = S (0.0936 ^{A})	C = S (0.5394 ^{A})	Oct 09 < June 10 (0.0000 ^{KW})	Oct 09 = June 10 (0.1315 ^{A})	KB > TB (0.0221 ^{KW})	KB > TB (0.0000 ^{A})

Kachemak Bay (KB). Thorndyke Bay (TB). S: MBP families from selected broodstock. C: MBP controls from unselected broodstock. Statistical differences ($P < 0.05$). KB = TB no significant difference detected between oysters grown at KB and TB. KB > TB (P-value) parameter is significantly greater in oysters grown at KB than at TB. Statistical test used: #.####^{A} = ANOVA and #.####^{KW} = Kruskal-Wallis test.

Table 3.6. Selected fatty acids and fatty acid classes of MBP oysters at KB and TB in June of 2010 (% total FA).

Family #	28	24	46	20	34	44	21	0	Mean	28	24	46	20	34	44	21	0	Mean
Location	**Kachemak Bay (AK)**									**Thorndyke Bay (WA)**								
SFA	23.6^{a}	23.5^{a}	24.2^{a}	22.3^{a}	23.9^{a}	21.5^{a}	23.1^{a}	24.5^{a}	23.3	29.2^{a}	22.9^{c}	24.2^{abc}	22.3^{bc}	23.8^{bc}	21.5^{ab}	23.1^{ab}	24.5^{ab}	26.3
	(0.5)	(0.4)	(0.2)	(0.3)	(0.0)	(2.5)	(0.2)	(0.1)	(0.5)	(0.3)	(0.3)	(0.2)	(0.3)	(0.0)	(2.5)	(0.2)	(0.1)	(0.8)
MUFA	19.1^{bc}	18.3^{c}	19.4^{bc}	22.8^{a}	19.4^{bc}	20.3^{abc}	21.8^{ab}	18.6^{c}	18.6	22.0^{ab}	20.5^{bc}	19.2^{a}	22.8^{c}	19.0^{a}	20.3^{ab}	21.8^{a}	18.6^{a}	22.2
	(0.4)	(0.2)	(0.5)	(0.1)	(0.2)	(1.4)	(0.3)	(0.3)	(0.3)	(0.4)	(0.1)	(0.5)	(0.1)	(0.2)	(1.41	(0.33	(0.3)	(0.3)
PUFA	50.8^{a}	50.0^{a}	48.5^{a}	50.3^{a}	50.3^{a}	51.7^{a}	51.1^{a}	50.1^{a}	50.1	42.2^{b}	49.9^{a}	48.5^{b}	50.3^{a}	50.3^{ab}	51.7^{b}	51.1^{b}	50.1^{b}	45.1
	(0.5)	(0.7)	(1.1)	(0.4)	(0.1)	(1.4)	(0.1)	(0.2)	(0.6)	(0.7)	(0.5)	(1.1)	(0.4)	(0.1)	(1.4)	(0.1)	(0.2)	(0.8)
ω-3	47.4^{a}	46.2^{a}	45.1^{ab}	46.0^{a}	46.9^{a}	48.2^{a}	42.2^{b}	46.6^{a}	46.3	39.2^{c}	46.9^{a}	45.2^{c}	46.9^{ab}	46.9^{bc}	48.2^{c}	42.2^{c}	46.6^{c}	41.9
	(0.5)	(0.7)	(1.1)	(0.4)	(0.1)	(1.2)	(0.1)	(0.1)	(0.5)	(0.7)	(0.5)	(1.1)	(0.4)	(0.1)	(1.2)	(0.1)	(0.13	(0.8)
ω-6	3.4^{a}	3.2^{a}	3.3^{a}	3.4^{a}	3.4^{a}	3.6^{a}	3.5^{a}	3.5^{a}	3.4	2.5^{a}	3.0^{c}	3.5^{c}	3.4^{ab}	3.4^{bc}	3.6^{bc}	3.5^{c}	3.5^{c}	2.7
	(0.0)	(0.0)	(0.1)	(0.0)	(0.0)	(0.2)	(0.1)	(0.1)	(0.1)	(0.0)	(0.0)	(0.1)	(0.0)	(0.0)	(0.2)	(0.1)	(0.1)	(0.1)
16:0	16.2^{a}	15.9^{a}	16.6^{a}	16.6^{a}	16.9^{a}	14.1^{a}	16.3^{a}	17.1^{a}	16.2	18.2^{a}	13.9^{c}	16.6^{abc}	16.5^{bc}	16.9^{abc}	14.1^{abc}	16.3^{abc}	17.1^{ab}	16.4
	(0.3)	(0.4)	(0.1)	(0.3)	(0.0)	(1.7)	(0.1)	(0.0)	(0.4)	(0.3)	(0.4)	(0.1)	(0.3)	(0.0)	(1.7)	(0.1)	(0.0)	(0.6)
20:5ω3	24.3^{a}	23.9^{a}	23.0^{a}	25.0^{a}	24.6^{a}	24.7^{a}	23.8^{a}	23.5^{a}	24.1	23.1^{b}	27.1^{a}	23.0^{b}	25.0^{a}	24.6^{b}	24.7^{b}	23.8^{b}	23.5^{b}	24.5
	(0.5)	(0.5)	(1.0)	(0.7)	(0.9)	(0.3)	(0.6)	(1.0)	(0.7)	(0.4)	(0.4)	(1.0)	(0.7)	(0.9)	(0.3)	(0.6)	(1.0)	(0.4)
22:6ω3	16.2^{a}	16.4^{a}	15.7^{a}	15.7^{a}	15.4^{a}	16.8^{a}	16.6^{a}	16.3^{a}	16.1	11.5^{c}	15.9^{a}	15.7^{c}	15.7^{ab}	15.4^{abc}	16.8^{abc}	16.6^{bc}	16.3^{c}	13.2
	(0.2)	(0.3)	(0.2)	(0.6)	(0.7)	(1.0)	(0.6)	(0.5)	(0.5)	(0.3)	(0.7)	(0.4)	(0.6)	(0.7)	(1.0)	(0.6)	(0.5)	(0.6)

Standard deviation of the mean (SD). Different superscript letters represent significant differences between families for each proximate component parameter (Tukey's Test, n = 3, 15 d.f., significant $P < 0.05$) differences were only tested for within sites and site interaction was not tested for families.

Table 3.7. Comparisons of selected fatty acids and fatty acid classes of MBP oysters samples in June of 2010 between top seven MBP selected families (S; selected at KB) versus a control family (C), and between KB and TB.

MBP Select vs. Control			
Site	**KB**	**TB**	**KB vs. TB**
SFA	S = C (0.1949^{A})	S = C (0.2801^{A})	KB < TB (0.0000^{KW})
MUFA	S = C (0.1454^{A})	S > C (0.0317^{A})	KB < TB (0.0000^{KW})
PUFA	S = C (0.7596^{A})	S = C (0.1020^{A})	KB > TB (0.0000^{KW})
ω-3	S = C (0.7435^{A})	S = C (0.1043^{A})	KB > TB (0.0000^{KW})
ω-6	S = C (0.5086^{A})	S = C (0.0648^{A})	KB > TB (0.0000^{KW})
16:0	S < C (0.0351^{A})	S = C (0.1743^{A})	KB = TB (0.3429^{KW})
20:5ω3	S = C (0.3987^{A})	S = C (0.1862^{A})	KB = TB (0.0000^{A})
22:6ω3	S = C (0.7819^{A})	S = C (0.1330^{A})	KB > TB (0.0000^{A})

Kachemak Bay (KB). Thorndyke Bay (TB). S: MBP families from selected broodstock. C: MBP controls from unselected broodstock. Statistical differences ($P < 0.05$). KB = TB no significant difference detected between oysters grown at KB and TB. KB > TB (P-value) parameter is significantly greater in oysters grown at KB than at TB. Statistical test used: $\#.\#\#\#\#^{A}$ = ANOVA and $\#.\#\#\#\#^{KW}$ = Kruskal-Wallis test.

Table 3.8. Fatty acid composition of MBP Oysters at KB and TB in June of 2010 (%w/w).

Family #	28	24	46	20	34	44	21	0	Mean	28	24	46	20	34	44	21	0	Mean
Location - Sample time	**Kachemak Bay (AK)**									**Thorndyke Bay (WA)**								
14:0	2.82	2.69	2.96	2.57	2.76	2.74	2.88	2.94	2.79	4.52	3.47	3.67	1.98	4.03	2.09	3.39	3.93	3.38
	(0.03)	(0.17)	(0.11)	(0.18)	(0.16)	(0.05)	(0.08)	(0.09)	(0.11)	(0.23)	(0.32)	(0.44)	(0.28)	(0.92)	(0.16)	(0.81)	(0.39)	(0.44)
18:0	3.66	3.05	3.37	3.93	3.77	1.92	3.15	3.43	3.29	4.17	4.21	4.01	3.96	3.76	4.58	3.27	3.80	3.97
	(0.17)	(1.00)	(0.23)	(0.11)	(0.15)	(0.09)	(0.07)	(0.02)	(0.23)	(0.18)	(0.06)	(0.55)	(0.16)	(0.09)	(0.13)	(0.29)	(0.05)	(0.19)
16:1ω7	2.98	3.33	2.91	2.62	2.91	2.92	3.23	2.90	2.98	4.38	4.19	4.21	2.03	4.32	1.88	4.04	4.80	3.73
	(0.31)	(0.23)	(0.31)	(0.13)	(0.29)	(0.06)	(0.17)	(0.32)	(0.22)	(0.14)	(0.01)	(0.10)	(0.35)	(0.17)	(0.22)	(0.15)	(0.31)	(0.18)
18:1ω11	1.98	1.65	1.94	2.16	1.56	5.56	1.82	1.91	2.32	1.45	1.50	1.78	1.81	1.63	2.10	1.49	1.43	1.65
	(0.05)	(0.70)	(0.19)	(0.08)	(0.56)	(0.20)	(0.12)	(0.07)	(0.24)	(0.07)	(0.08)	(0.24)	(0.01)	(0.44)	(0.18)	(0.14)	(0.28)	(0.18)
18:1ω9 *cis*	3.84	4.27	4.37	3.96	4.07	3.94	3.96	3.81	4.03	3.41	3.17	3.60	2.96	3.74	2.79	3.77	3.76	3.40
	(0.09)	(0.94)	(0.66)	(0.35)	(0.24)	(0.21)	(0.22)	(0.34)	(0.38)	(0.46)	(0.21)	(0.40)	(0.06)	(0.23)	(0.08)	(0.25)	(0.15)	(0.23)
18:1ω7	5.59	5.96	5.21	5.42	5.30	5.56	5.80	5.27	5.51	6.41	6.33	7.26	6.60	6.50	6.66	7.23	6.85	6.73
	(0.37)	(0.44)	(0.40)	(0.09)	(0.10)	(0.20)	(0.33)	(0.42)	(0.29)	(0.14)	(0.01)	(0.10)	(0.35)	(0.17)	(0.22)	(0.15)	(0.31)	(0.18)
18:2ω6 *cis*	2.36	2.44	2.27	2.16	2.38	2.28	2.40	2.36	2.33	1.21	1.08	1.21	1.18	1.24	1.16	1.23	1.11	1.18
	(0.04)	(0.33)	(0.11)	(0.05)	(0.04)	(0.11)	(0.05)	(0.14)	(0.11)	(0.02)	(0.05)	(0.03)	(0.10)	(0.04)	(0.06)	(0.03)	(0.02)	(0.05)
18:3ω3	1.86	1.79	1.78	1.71	1.76	1.66	1.89	1.89	1.79	1.08	0.73	0.85	0.78	0.96	0.77	0.90	0.77	0.86
	(0.13)	(0.27)	(0.16)	(0.04)	(0.08)	(0.03)	(0.19)	(0.26)	(0.14)	(0.17)	(0.09)	(0.03)	(0.09)	(0.09)	(0.04)	(0.07)	(0.06)	(0.08)
18:4ω3	5.03	4.83	4.74	4.69	4.38	4.54	5.09	4.94	4.78	3.52	2.75	3.33	3.05	3.21	3.32	3.62	3.16	3.24
	(0.38)	(0.58)	(0.50)	(0.17)	(0.25)	(0.17)	(0.24)	(0.59)	(0.36)	(0.04)	(0.22)	(0.10)	(0.27)	(0.02)	(0.25)	(0.01)	(0.07)	(0.12)

Standard deviation of the mean (SD).

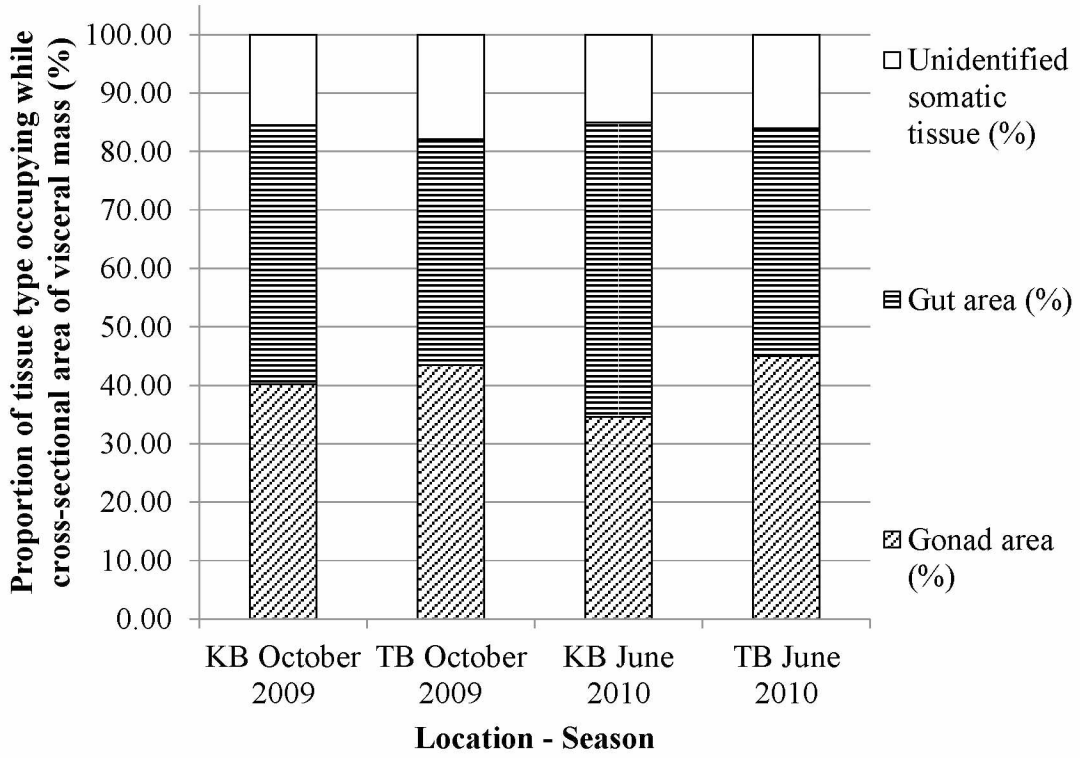

Figure 3.3. Percent area composition (gonad, gut and unidentified somatic tissues) of cross-sectional area of whole visceral mass of oyster sections by sampling location and season.

Table 3.9. Comparisons of reproductive condition (gonad area) of MBP oysters between top seven MBP selected families (S) versus a control family (C), between sites, and between seasons.

Interaction	Site * season * control				Season		Site	
Location / Sample time	**KB Oct - 09**	**TB Oct - 09**	**KB June - 10**	**TB June - 10**	**Oct - 09**	**Jun - 10**	**KB**	**TB**
Comparison	**Control (C) vs. 7 selected MBP families (S)**				**KB vs. TB**		**Oct - 09 vs. Jun - 10**	
Total gonad area (%)	C = S (0.1727 ^{A})	C = S (0.0923 ^{A})	C = S (0.1752 ^{A})	C = S (0.1727 ^{A})	KB < TB (0.0202 ^{KW})	KB < TB (0.0000 ^{A})	Oct-09 > Jun-10 (0.0001 ^{A})	Oct-09 = Jun-10 (0.8810 ^{KW})

Kachemak Bay (KB). Thorndyke Bay (TB). S: MBP families from selected broodstock. C: MBP controls from unselected broodstock. Statistical differences ($P < 0.05$). KB = TB no significant difference detected between oysters grown at KB and TB. KB > TB (P-value) parameter is significantly greater in oysters grown at KB than at TB. Statistical test used: #.####^{A} = ANOVA and #.####^{KW} = Kruskal-Wallis test.

Chapter 4: General Conclusions

Differences in environment combined with selection of families from MBP cohorts resulted in differences in growth, survival, shell shape, meat content, reproduction, and biochemical composition for genetically identical families planted at growout sites at both KB and TB.

By the comparison of the growth and biometric characteristics of the top seven MBP Cohort 20 families at KB to the same families grown at TB, we can conclude that oysters selected as generalists at lower latitudes such as Washington (WA) do not necessarily perform as generalists when grown at the higher latitude of Alaska (AK). Latitudinal differences and significantly cooler water conditions at KB meant that oysters grown using suspended culture grew more slowly and were generally smaller after three years of growth than those oysters grown inter-tidally at TB. At KB, oysters had significantly lower values for whole weight, shell weight, meat weight, cavity volume, length, width, and depth than TB oysters. Standardization of biometric data using condition indices was used to eliminate the effect of size when comparing multiple oyster dimensions, generating a more complex description of shape, condition, and quality.

It was concluded that external influences such as handling, mechanical hydro-action, and different temperature regimes played a significant role in development and differences in shell shape. There were no differences in CI^E indicating that shell shapes were of similar and equally desirable dimensions, with oysters at both KB and TB exhibiting good depth relative to both width and length, despite the large discrepancy in size distributions of oysters between the two sites.

Selected MBP families had greater meat fill than the control family of oysters when sampled at the end of summer in October of 2009 at both KB and TB. However, overwintering

was equally demanding on both selected and unselected oysters, as cavity fill was similar between selected and control MBP oyster families. KB oysters were slower to recover meat condition than oysters grown at the lower latitudes of TB and this is likely due to cooler water temperatures and the prolonged winter in AK. Changes and differences in meat condition of the oysters relative to selection, environment, and culture type at both sites were reflected in patterns and changes in biochemical composition.

Selection generally did not act to increase the content of any biochemical components, with the exception of protein and lipid content at KB in October of 2009 and TB in June of 2010. As expected, the solid content of oysters correlated with CI^{HN} between sites and sampling times. The degree of reproductive development occurring in the oysters at either sample site was dependent on water temperatures, and had a distinct effect on the biochemical composition of the animals. The lower temperatures at KB inhibited reproductive development, and as a result the oysters did not metabolize as much glycogen relative to their counterparts at TB. Oysters at KB also appeared to conserve glycogen at the cost of protein as an adaptive response to cool temperatures as an energetic reserve for gametogenesis, a relationship that was not observed at TB. Cold conditions characteristic of the region were also responsible for higher levels of omega-3 and omega-6 fatty acids, particularly in the form of DHA at KB compared to TB. In terms of perceived quality this meant that the extra-small and small oysters at KB likely taste sweeter as a function of higher glycogen levels and possessed higher concentrations of fatty acids known to improve a number of health characteristics. In contrast, oysters from TB reach market size faster and from nutritional standpoint maintain a protein content. Finally a number of MBP Cohort 20 families performed consistently well with regards to a number of parameters; this was particularly true of MBP Cohort 20 Family 28, the top yielding at KB in October of 2009.